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SOIL SCIENCE

Editor-in-Chief
JACOB G. LIPMAN

Associate Editor
HERMINIE BROEDEL KITCHEN

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A Method for Measuring Carbon Dioxide Evolution from Soil. HARRY HUMFELD.....	1
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SOIL SCIENCE



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A METHOD FOR MEASURING CARBON DIOXIDE EVOLUTION FROM SOIL¹

HARRY HUMFELD²

Bureau of Chemistry and Soils, U. S. Department of Agriculture

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In connection with studies on the microbiological activities in soils of different reaction as affected by the addition of green manures, it was deemed desirable to obtain some information on the rate of carbon dioxide evolution. A search of the literature for a suitable method for the determination of carbon dioxide from the soil revealed that, in general, the methods used may be divided into a number of distinct groups:

1. The soil sample is taken in a way intended to prevent the diffusion of air or to reduce it to a minimum. The sample is brought to the laboratory and analyzed for carbon dioxide content. The work of Leather (1) is an example of this type.

2. The soil air in a measured quantity is obtained from the soil in situ by means of a tube thrust or placed into the soil and the air obtained is analyzed in the laboratory. Russell and Appleyard's (6) and Potter and Snyder's (5) experiments illustrate this group of methods.

3. Soil samples are taken, brought into the laboratory, and carbon dioxide evolution is measured by placing the soil in a flask and passing carbon dioxide-free air over it. This method, used by Waksman and Starkey (7) and by many others, is essentially a measure of the carbon dioxide producing power of the soil under certain given conditions.

4. Marsh (4) modifies the preceding method in such a way as to pass air through the soil as well as over it.

5. Lundegardh (2) enclosed a sample of soil in a flask and measured the carbon dioxide accumulation in the flask air after 24 hours.

6. Lundegardh (3) later says that, methods which determine the carbon dioxide as it escapes from the soil by means of diffusion are in general, to be preferred to methods where an air stream passing through the soil is used. He therefore evolved an apparatus which consists of a "respiration bell" that is placed over an area of soil to collect the carbon dioxide as it escapes. At the end of 20 minutes a sample of the air under the bell is taken and analyzed. This, no doubt, gives results which approach natural conditions more closely than any of the other methods mentioned.

According to Lundegardh the rate of diffusion under these conditions is proportionate to time. Assuming that this is true, in soils of low organic activity, it very likely would not necessarily be true if the carbon dioxide evolution were considerably higher.

In our studies on decomposition of green manures under greenhouse conditions, preliminary tests showed that the amount of carbon dioxide evolution was 10, or more, times as great as that from untreated soil. Since it was be-

¹ Paper 332 of the outside publication series, Bureau of Chemistry and Soils.

² Assistant bacteriologist, office of soil microbiology.

lieved that such a rapid accumulation of carbon dioxide under the bell would tend to decrease the rate of diffusion, it seemed preferable to develop a method that would remove the carbon dioxide as fast as it diffused from the soil.

THE APPARATUS

The apparatus as finally developed consisted, therefore, of three essential parts—a collecting unit, an absorbing unit, and a source of suction.

The collecting unit

A rectangular galvanized iron box, 8 inches long, 3 inches wide, and 3 inches high, was used as a modified form of Lundegard's "respiration bell," small enough for use on the greenhouse plots. At each end of the box equidistant from the sides, a tube $\frac{1}{4}$ inch inside diameter, was inserted. One tube served for inlet, the other for outlet. The open side of the box was pressed down into the soil about one inch.

The absorption unit

The absorption unit consisted of a train of four bottles preceded by a flow-meter, which, in turn, consisted of a piece of capillary tubing 8 inches long and $\frac{1}{2}$ to 1 mm. in inside diameter, fastened in a U-shaped piece of glass tubing, 5 mm. in diameter, as shown in plate 1, figure 1. The flow-meter was fastened to a piece of wall board 12 inches square, and the U-shaped glass tubing was half filled with a colored mineral oil. The flow-meter was then calibrated in liters per hour against a West test meter. The flow-meter was connected with a piece of rubber tubing to the first bottle.

Wide-mouthed, approximately 1-liter bottles were used. The mouths were closed with two-hole rubber stoppers, each hole being fitted with a piece of glass tubing bent at right angles. The first bottle was empty and was used as a safeguard against dilution of the absorbent solution with moisture condensing from the air after passage over the soil. The second and third bottles each contained 800 cc. of 0.08 to 0.16 *N* KOH. Each of the absorption bottles was connected with a patented gas distributor consisting of a glass tube in which a disc made of ground sintered glass is fitted in such a way that the suction breaks up the air passing through the solutions into a great number of small bubbles. This device was found to be very efficient in promoting complete absorption. The fourth bottle was empty and served as a safeguard. It seemed also to facilitate adjustment of the rate of flow, since the air in this bottle acted as a cushion against rapid changes. The flow of air was regulated by means of a glass stopcock fastened in the line after the fourth bottle. A very delicate adjustment, however, was necessary.

The source of suction

A vacuum pump operated by an electric motor was used. A water aspirator was also found to give satisfactory results. Any number of units up to the

capacity of the pump could be connected together so that carbon dioxide evolution could be studied on a number of plots simultaneously.

As stated, the "respiration box" is placed in a suitable position on the soil. In order to obtain air of uniform carbon dioxide content, a rubber tube, 3 to 4 feet long, was fastened to the inlet of the box and tied up, with the opening about 3 feet above the surface of the soil. The outlet tube was connected by means of rubber pressure tubing to the calibrated flow-meter, which in turn was fastened by the same means to the glass tube in the first bottle. The bottles were connected together, the stoppers of the two bottles containing the KOH solution being provided with a glass trap. A form of trap used in the distillation apparatus for Kjeldahl nitrogen analyses was found satisfactory to prevent any mechanical carrying over of solution into the next bottle. The glass tube from the last bottle was then connected to the glass stopper in the suction line.

THE USE OF THE APPARATUS

It was found that 10 liters of air an hour was sufficient to carry off the carbon dioxide from the soil, even at the highest rate of evolution encountered. Lower rates could be used with smaller amounts of carbon dioxide, but it was found that with smaller rates of flow the adjustment to keep the flow steady was more difficult.

As the apparatus was used, the KOH solution in the first bottle absorbed all of the carbon dioxide, the second bottle acting as a check on the completeness of absorption. When it was found that the carbon dioxide had neutralized the KOH solution to such an extent that the next absorption period would reduce the strength to less than one-half the original strength, a bottle containing new KOH solution was inserted in its place. As a rule, samples were taken and titrated every 24 hours.

After a number of variations, the titration was finally standardized so that now it is as follows: hydrochloric acid was made up of such a normality (0.08336 N) that 1 cc. was equivalent to 0.5 mgm. carbon as carbon dioxide. The KOH solution was made up to approximately the same strength or any strength up to twice this strength, depending upon the rapidity of carbon dioxide evolution expected. Each 24 hours a 25-cc. aliquot was taken from each bottle in each unit.

These aliquots were brought to boiling and titrated first to the endpoint of phenolphthalein and then to the endpoint of methyl orange. Aliquots of the original KOH solution were titrated in a similar manner and the amount of the titration from the endpoint of phenolphthalein to the endpoint of methyl orange was subtracted from the corresponding titration of the aliquots taken after absorption. The number of cubic centimeters of hydrochloric acid required, divided by 2, gave the milligrams of carbon as carbon dioxide in the aliquot. From this, the carbon dioxide evolution for the area of the box or for any unit area could be calculated. It was found that dilution of the ali-

quots with 100 cc. distilled water before heating gave better endpoints for phenolphthalein.

In order to eliminate any possibility of suction of air from the soil, instead of freeing the air from carbon dioxide before passing it over the soil, a blank determination was run. The net amount of carbon dioxide obtained from the soil was then determined by deducting the amount of this blank determination. The calculation then was as follows:

The number of cubic centimeters of hydrochloric acid for the phenolphthalein titration is deducted from the number of cubic centimeters required for the

TABLE 1
Typical titrations for a 24-hour period on plots in the greenhouse^{} under different treatments*
(C as CO₂ = mgm. in 24 hours)

PLOT NUMBER	GREEN MANURE USED	HCl REQUIRED†		DIFFERENCE MINUS BLANK TITRATION‡ C AS CO ₂	C AS CO ₂ PER BOX; IN 24 HOURS
		Phenolphthalein	Methyl orange		
		cc.	cc.	mgm.	mgm.
L 1	None	40.0	44.7	1.3	17.2
L 2*	None	39.6	44.7	1.5	24.0
L 3	Rye	31.3	44.7	5.7	106.8
L 4*	Rye	36.9	44.7	2.9	67.2
L 5	None	40.2	44.7	1.2	14.4
L 6*	None	37.6	44.6	2.4	51.2
L 7	Vetch	37.5	44.7	2.6	57.6
L 8*	Vetch	35.4	44.7	3.6	91.2
L 9	Rye	36.5	44.7	3.1	73.6
L 10*	Rye	34.6	44.6	4.0	102.4
L 11	Vetch	38.0	44.7	2.3	49.6
L/12*	Vetch	35.4	44.7	3.6	91.2
Air	Control	44.1	44.7	0.8	25.6§

* All even-numbered plots had been limed and were neutral in reaction, whereas the odd-numbered plots were acid.

† 25-cc. aliquots titrated.

‡ Blank titration equals 2 cc.

§ This figure has been deducted from each preceding figure in this column.

methyl orange titration. This gives the number of cubic centimeters actually used by the carbon dioxide absorbed.

The original KOH solution, however, required a certain amount of hydrochloric acid to change the reaction from the endpoint of phenolphthalein to the endpoint of methyl orange. This amount, which was 2 cc. in the KOH solution used, is deducted from the apparent difference. The figures are given in the column headed "Difference minus blank titration," table 1. This figure, multiplied by the number of cubic centimeters of KOH solution in the absorption bottle and divided by half the number of cubic centimeters in the aliquot taken for titration, gives the milligrams of carbon dioxide absorbed from the area of soil covered by the respiration box.

For plot L4, in table 1, the number of milligrams of carbon dioxide would be

$$\frac{44.7 - 40.0 - 2.0}{2} \times \frac{800}{25} = 43.2$$

If it is desired, the milligrams of carbon dioxide absorbed from the carbon dioxide in the air, given in the last line of table 1, can be deducted. The net results in this case would be

$$43.2 - 26.0 = 17.2 \text{ mgm. carbon dioxide.}$$

The method described was used in the determination of carbon dioxide evolution under greenhouse conditions.

FIELD EXPERIMENTS

Subsequently it was decided that trials with the same apparatus under field conditions would be advisable. Accordingly the apparatus was set up in the field on some experimental plots on Keysport clay loam. Two units were placed on each of three plots. One plot was kept fallow, another plot had a heavy growth of soybeans, and the third plot was growing a crop of corn broadcast for green manure. At the start of the carbon dioxide determination the crops were fully grown and ready to be turned under. The soil was exceedingly dry, however, and it was considered inadvisable to turn the corn and soybeans under before a rainfall had occurred. The results obtained for a number of days therefore indicate the amount of carbon dioxide given off by the soil under very low moisture content.

A rain occurred on September 5 and 6 and the crops were plowed under on September 7. However, the moisture in the soil was still far from optimum, therefore, the table given also shows the time and amount of rainfall during the experiment.

The first data obtained showed that only small amounts of carbon dioxide were being given off. It was essential, therefore, that chemical determination be as accurate as possible. The concentration of KOH was therefore reduced so that it was only about 0.04 to 0.05 normal.

When the titrations were made it was noticed that the endpoint of phenolphthalein was too indefinite, because of the loss of carbonate from the solution as carbon dioxide; this caused the reappearance of the red color of phenolphthalein. In order to overcome this difficulty the carbonate in the solutions was precipitated as barium carbonate by adding an excess of barium chloride to the samples taken for titration.

The procedure was standardized as follows:

Two hundred cubic centimeter of each sample were placed in tall glass cylinders, an excess of approximately normal barium chloride (5 to 20 cc. was usually required) was added, and the volume made up to 250 cc. with distilled water. The cylinders were then stoppered and the solutions were shaken. Time was allowed for the precipitate to settle and when the supernatant solution was clear two 25- or 50-cc. aliquots were pipetted off and titrated.

The original KOH solution was treated in a similar manner and the number of cubic centimeters of HCl required for the original KOH solution minus the number of cubic centimeters required for the solutions after CO₂ was passed through them, gives the number of cubic

TABLE 2

*Amount of carbon as carbon dioxide given off in 24 hours from soil under different treatments—
September 24, 1929*

PLOT NUMBER	TREATMENT	BOTTLE	ALiquot TAKEN FOR TITRATION	HCl REQUIRED		DIFFERENCE USED BY CO ₂	C AS CO ₂ FOR AREA OF BOX	MINUS AMOUNT IN AIR	C AS CO ₂ PER SQ.M.	RATE OF AIR HOURLY
					Average					
			cc.	cc.	cc.	cc.	mgm.	mgm.	gm.	liters
K6W	Soybeans	1	25	33.25	33.22					
			25	33.20		5.48	109.6	54.4	3.52	13
		2	25	38.72	38.70	0.00	0.0			
			25	38.68						
K6W	Soybeans	1	25	33.20	33.15	5.55	111.0	57.0	3.68	13
			25	33.10						
		2	25	38.60	38.64	0.06	1.2			
			25	38.68						
K4E	Fallow	1	50	15.99	15.05	6.15	61.5	31.8	2.05	7
			50	16.11						
		2	50	21.10	21.20	0.00	0.0			
			50	21.29						
K4E	Fallow	1	50	14.60	14.66	6.54	65.4	27.2	1.76	9
			50	14.72						
		2	50	15.65	15.74	0.00	0.0			
			50	15.82						
K7E	Corn	1	25	33.29	33.14	5.56	111.2	59.8	3.87	13
			25	32.99						
		2	25	38.55	38.55	0.15	3.0			
			25	38.54						
K7E	Corn	1	25	32.72	32.74	5.96	119.2	67.8	4.38	13
			25	32.77						
		2	25	38.53	38.56	0.14	2.8			
			25	38.60						

centimeters of HCl equivalent to the CO₂ absorbed. These figures, multiplied by the proper factor to obtain the amount in the total amount of solution and divided by 2, since each cubic centimeter of HCl is equivalent to 0.5 mgm. of C as CO₂, give the mgm. of C as CO₂ obtained from the area of soil under the respiration box.

The number of mgm. for the area under the box multiplied by 64.6 and divided by 1,000 gives the number of grams of carbon as carbon dioxide given off by each square meter of soil area.

Table 2 gives an example of a typical series of titrations, and shows the accuracy of the method. Results show that the greatest variations obtained on duplicate boxes on the same plots are due to differences caused by activities in the soil and that as far as the analytical operations are concerned, very good results can be obtained.

Table 3 shows what results may be obtained under field conditions, as described, when green manure is turned under. This table shows the amount of carbon as carbon dioxide given off, both before and after the green manures were turned under, and the effect of the decomposition of soybeans and of corn on the carbon dioxide evolution.

The crop was rolled down flat and plowed under as would be done under actual field operations. The respiration boxes were then placed on the surface of the soil at random so that any differences in determinations would be due to variations in the amount and nature of the organic matter below them.

The results show that although in a general way the fallow plot gives fairly good agreements between duplicates, the results obtained from duplicates on the soybean and the corn plots show considerable differences after the crops were turned under.

As moisture in this case was no doubt a limiting factor, an increase was shown in carbon dioxide evolution wherever a rainfall occurred in sufficient amount to cause a definite increase in the soil moisture. This is true of the fallow plots as well as of the soybeans and the corn plots.

In adapting the method to field conditions, it was found that it was impossible to keep the flow of air in all the units at 10 liters an hour, without variation. This difficulty was partly overcome by using a safety valve, consisting of a large glass test tube partly filled with mercury and fitted with a 2-hole rubber stopper provided with two bent glass tubes, one of which reached down into the mercury. This safety valve was connected with a bent glass tube fitted into the stopper of the fourth bottle in the absorption unit, that is, the bottle next to the source of suction.

The rate was then more satisfactorily regulated by opening the stopcock in the suction sufficiently to allow a somewhat greater amount of air to pass than the required 10 liters an hour. Then by moving the long glass tube in the safety valve up or down the rate could be cut down to the required amount. Some variation still took place during the 24 hours between samplings, but adjustments could be made much more readily.

It had been observed, also, that as the air passed from the respiration box, through the flow-meter and into the absorbing bottles, a certain amount of moisture condensed, especially in the flow-meters. This diluted the oil in the flow-meters and necessitated renewal of the oil from time to time.

In order to overcome this an empty bottle was inserted between the box and the flow-meter. Indications are that this modification eliminates the moisture accumulation or at least reduces it to a minimum.

Plate 1, figure 3 shows the absorption unit as it is now used in the field.

The method as now employed seems to be adapted to the measurement of carbon dioxide evolution from the soil or from material incorporated with the soil, assuming that diffusion from the soil is a direct result of activities resulting in the liberation of carbon dioxide.

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PLATE 1

THE CARBON DIOXIDE APPARATUS

FIG. 1. A unit of the carbon dioxide apparatus.

FIG. 2. General view of greenhouse plots and units of carbon dioxide apparatus.

FIG. 3. A Unit of the Carbon Dioxide Apparatus Set up in the Field.

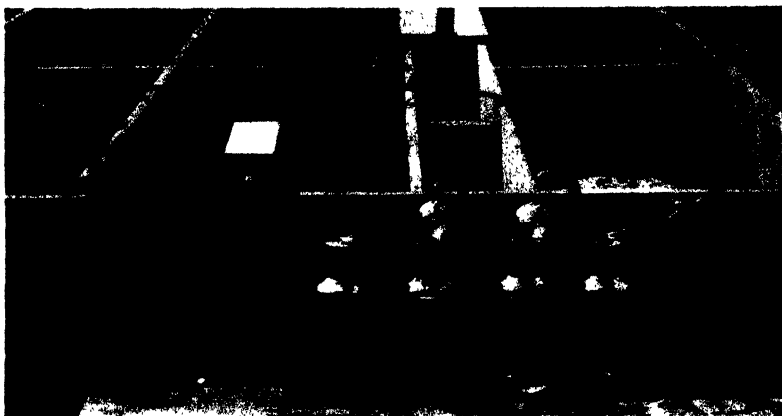


FIG. 1



FIG. 2

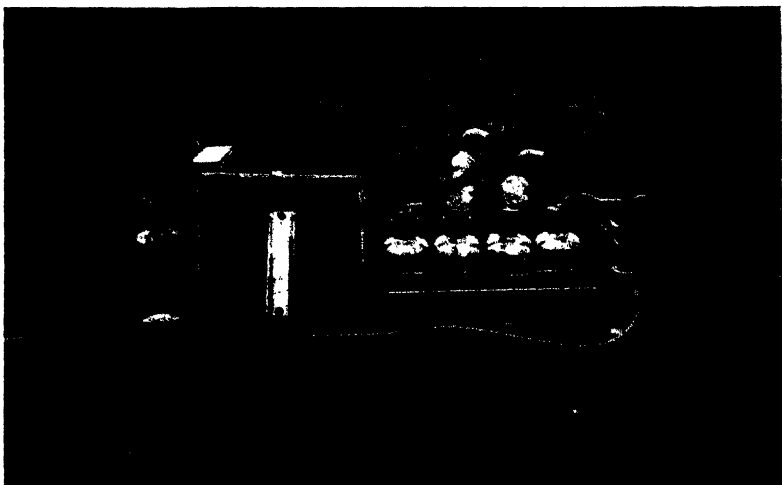


FIG. 3

PHOSPHATE STUDIES IN SOLUTION CULTURES¹

J. W. TIDMORE²

Alabama Agricultural Experiment Station

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The question of minimum phosphate concentration for maximum plant growth should be of particular interest since there are many soils reported which have extremely low phosphate concentration in the soil solution as obtained by the method described by Burd and Martin (1). Parker and Tidmore (9) and Pierre and Parker (12) cited soil solutions containing as little as 0.02 p.p.m. inorganic PO_4 , and some of these soils produced in the field as much as 45 bushels of corn an acre.

Considerable work has been done during the past few years concerning the minimum concentration of phosphate required for maximum growth of plants in solution cultures. Hoagland and Martin (4) obtained a satisfactory growth of barley in culture solutions containing 0.7 p.p.m. PO_4 , and a maximum growth at 1.1 p.p.m. PO_4 . Parker (8) obtained maximum growth of corn and soybeans in culture solutions having a phosphate concentration of 0.5 p.p.m. Subsequently, Parker and Pierre (11) obtained a maximum growth of corn at a phosphate concentration of 0.25 p.p.m. They expressed the opinion that a very good growth could be obtained at a phosphate concentration of 0.1 p.p.m., if that concentration could be actually maintained.

In the culture solution experiments mentioned in the foregoing, the phosphate concentrations were not adequately maintained. The quantity of the culture solution used for each culture was entirely too small for the number of plants grown in it. Hoagland and Martin (4) used 4-liter containers for one plant whereas Parker and Pierre (11) used 100-liter vessels for three plants. They state that with that volume the PO_4 concentration was not adequately maintained. It seemed desirable, therefore, to make a further study of plant growth in culture solutions of varying phosphate concentrations.

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² Associate professor of agronomy and soils, Alabama Agricultural Experiment Station.

In the following experiments a very large volume of solution with relatively few plants was used for each culture. The phosphate concentration was practically maintained. The following points were studied in the investigation:

1. The rate of phosphate absorption by plants grown in solutions of various phosphate concentrations.
2. The influence of low phosphate concentrations on the total plant growth and on the rate of growth.
3. The phosphate concentration and buffer capacity of the sap of plants grown in solutions of various phosphate concentrations.
4. The influence of the reaction of the culture solution on growth and on phosphate absorption.

EXPERIMENTAL PROCEDURE

Phosphate concentrations of 0.05, 0.1, 0.2, and 0.5 p.p.m. were maintained constant in the various cultures by using a large volume of solution for each plant and renewing the PO_4 content of the solution from one to three times a day. Nine plants were grown in a 1,000-liter culture vessel. To continually

TABLE 1
Composition of the culture solution

	K	Ca	Mg	NO_3	SO_4	B	NaCl	MnSO_4
Mgm. equivalent.....	0 76	1.90	0 81	2.61	0.81
P.P.M.....	29	38.17	9 86	162 1	39 0	0.25	0.60	0.375

renew the solution about the plant roots and to aerate the cultures, the solution was thoroughly stirred by bubbling a strong stream of air through it.

In order to renew the phosphate concentration of the culture solutions, the PO_4 was determined by using a small aliquot of each solution; then sufficient potassium acid phosphate was added to give the desired PO_4 concentration. During the growing period, the culture solutions with 0.05, 0.10, 0.20, and 0.50 p.p.m. PO_4 had an average minimum concentration of 0.048, 0.086, 0.147, and 0.440 p.p.m. PO_4 , respectively, as determined by the colorimetric method described by Parker and Fudge (10).

The culture solution used in the experiments was one-fourth the concentration of Hoagland's (4, p. 372) with the addition of a small amount of boron. Table 1 gives the composition of the culture solution. Iron was supplied as ferric tartrate, as described by Parker (8). The plants were given iron about once each week, or whenever a slight chlorotic condition indicated a deficiency.

In experiment 1, the height of the plants, measured from the base of their stem to the tip of the longest leaf, was determined each week to indicate the rate of growth. The green weight of the plants was also obtained each week in experiment 2 after removing the plants from the culture solutions and allowing the root system to drain.

After the plants were harvested, they were weighed and a portion was placed in a cold storage room (20°F.) for freezing so that the plant sap could be obtained by means of a screw press. The concentration of PO_4 in the sap was determined and buffer curves were made. After the dry weight of the plants was determined, they were ground and the percentage PO_4 was determined by the use of Fiske and Subbarow's method as described by Parker and Fudge (10).

EXPERIMENT 1

Corn, sorghum, and tomato seeds were germinated in quartz sand and transferred, when 1 week old, to the culture solutions containing different phosphate concentrations. Three seedlings of each crop were grown in each culture solution for 46 days. Each treatment was in duplicate.

While the plants were being grown in the culture solutions, similar plants were grown in 4-gallon soil cultures of a sandy loam fertilized at the rate of

TABLE 2
 PO_4 absorbed, during one week, by plants growing in culture solutions of the phosphate concentrations indicated

ABSORPTION PERIOD	PO_4 (P P M) IN CULTURE SOLUTIONS			
	0 05	0 1	0 2	0 5
<i>weeks</i>	<i>mgm</i>	<i>mgm</i>	<i>mgm.</i>	<i>mgm.</i>
3	32	60	260	440
4	57	214	460	1,060
5	34	328	633	1,236
Total.....	123	602	1,352	2,736

1,000 pounds of superphosphate, 100 pounds of muriate of potash, and 500 pounds of sodium nitrate to the acre. There were duplicate cultures with two plants each.

Rate of PO_4 absorption

The amount of PO_4 absorbed from each culture solution was determined for three weeks, beginning when the plants were 3 weeks old. The results, given in table 2, represent the average of duplicate cultures, which agreed rather closely. It may be seen from table 2 that the phosphate absorbed is almost directly proportional, with few exceptions, to the concentration of PO_4 in the culture solution. The plants were larger and had a more extensive root system in the culture solutions at higher phosphate concentrations. This would account, in part, for an increased PO_4 absorption as the phosphate concentration of the culture solution increased.

Rate of growth and crop yields

The average height of these plants was determined each week during the growing period beginning when the plants were about 3 weeks old. The weekly increase in height is shown in table 3. The rate of growth, as measured by the height of the plants, grown in solutions having a phosphate concentration of 0.1, 0.2, and 0.5 p.p.m. PO_4 is about the same. Corn plants grown at 0.2 and 0.5 p.p.m. PO_4 increased in height each week an average of 35 cm., whereas those at 0.1 p.p.m. increased 28 cm. This difference in rate of growth is probably insignificant. This method of determining the rate of growth may be misleading because the diameter of the stems increased with the PO_4 concentration of the culture solution. It would seem that a determination of the weight of the plants at intervals would give a better indication of the rate of

TABLE 3

Average weekly increase in height of the plants grown in culture solutions at PO_4 concentrations indicated

AGE OF PLANTS	CORN				SORGHUM				TOMATO			
	PO_4 (p.p.m.) in culture solution				PO_4 (p.p.m.) in culture solution				PO_4 (p.p.m.) in culture solution			
	0.05	0.10	0.20	0.50	0.05	0.10	0.20	0.50	0.05	0.10	0.20	0.50
weeks	cm.	cm.	cm.	cm.	cm.	cm.	cm.	cm.	cm.	cm.	cm.	cm.
2	30	36	41	57	14	16	18	24	8	11	11	13
3	11	24	35	37	8	23	24	27	5	7	12	10
4	11	28	31	32	9	19	27	22	6	11	11	10
5	21	28	39	34	11	23	30	27	8	12	11	14
6	19	34	34	41	15	41	50	59
Average...	15.5	28.5	34.7	36.0	10.7	26.5	32.7	33.7	6.3	10.0	11.3	11.3

growth. The corn plants in culture solutions containing 0.05 p.p.m. PO_4 increased in height each week only 15 cm. This shows that the corn plants at 0.05 p.p.m. PO_4 made a much slower growth than did those at higher concentrations. The rate of growth in each culture was practically constant, as determined by measuring the plants. The same general trend holds for sorghum.

The increase in height of tomato plants each week was 6, 10, 11, and 11 cm., grown in culture solutions containing 0.05, 0.1, 0.2, and 0.5 p.p.m. PO_4 , respectively.

The most interesting result of this experiment is set forth in table 4, which gives the dry weights of the plants grown at various phosphate concentrations. To obtain these weights the plants were harvested and dried in an oven at 70°C . for two days. Three plants were weighed together, because the roots were entangled and separation was not feasible. Table 4 shows the average weight of three plants; the duplicates agreed as well as could be expected.

Contrary to the findings of Parker and Pierre (11), in this study the corn plants which were grown in culture solutions having a PO_4 concentration of 0.2 p.p.m. weighed about 65 per cent as much as those grown at 0.5 p.p.m. PO_4 . Corn made very little growth in solution cultures with 0.05 p.p.m. PO_4 . It will be noticed that the corn (roots and tops) grown at 0.05 p.p.m. weighed approximately one-third as much as those grown at 0.1 p.p.m. PO_4 . The data indicate a decided increase in corn growth as the phosphate concentration of the culture solution increased. The top:root ratio increased with the phosphate concentration of the culture solution. This would indicate that the root growth was relatively larger than the top growth at low phosphate concentrations.

As shown in table 4, corn grown in the soil cultures, which were well fertilized, made very little more growth than those grown in the culture solutions at 0.1 p.p.m. PO_4 . The root growth was smaller in the soil cultures than in the culture solutions at 0.1 p.p.m. PO_4 . Of course, the weight of the root system

TABLE 4

Dry weight of 3 plants grown in culture solutions with PO_4 concentrations indicated

PO_4	CORN			SORGHUM			TOMATOES		
	Tops	Roots	Total	Tops	Roots	Total	Tops	Roots	Total
p.p.m.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.
0.05	10.9	7.1	18.0	7.0	4.1	11.1	10.6	4.3	14.9
0.10	38.7	19.2	57.9	26.0	9.9	35.9	31.8	7.7	39.5
0.20	84.2	24.6	108.8	42.1	11.5	53.6	40.5	9.8	50.3
0.50	136.9	30.4	167.3	48.0	12.9	60.9	43.2	7.8	51.0
Soil	55.2	13.4	68.6	32.9	5.6	38.5	49.8	9.1	58.9

may not necessarily be a measure of the absorbing surface. The table shows that sorghum and tomatoes made as good growth in culture solutions at 0.2 p.p.m. as at 0.5 p.p.m. PO_4 . In most cases the differences are too small to be significant. It is claimed that these crops require a large amount of phosphate, yet they practically made a maximum growth at 0.2 p.p.m. PO_4 . The growth of sorghum and tomatoes in soil cultures was about the same as in culture solutions at 0.2 p.p.m. PO_4 .

PO_4 content of plant and plant sap

After the plants were dried and ground, the percentage PO_4 was determined by the Fiske and Subbarow method, as described by Parker and Fudge (10). The results, given in table 5, show that in general the PO_4 content of the plants increases with increasing concentrations of PO_4 in the culture solutions. The magnitude of the increase was greatest in sorghum and smallest in tomatoes. With all three crops the increase in PO_4 content was greater in the tops

than in the roots. In the case of corn and sorghum grown at concentrations of 0.05 and 0.1 p.p.m. PO_4 , the PO_4 content of the roots was higher than that of the tops. The opposite relation existed when the PO_4 concentration of the culture solution was increased to 0.2 and 0.5 p.p.m. At all concentrations of PO_4 the roots of the tomato plant had a higher percentage of PO_4 than the tops.

It is of interest to note that the PO_4 content of the tomato plants was uniformly much higher than that of the corn and sorghum. This might be expected to indicate that the tomato would make poorer growth in solutions of low PO_4 content than would corn or sorghum. Such, however, is not the case, as is indicated by the yields recorded in table 4.

TABLE 5

PO_4 content of dry tissue of plants grown in culture solutions with PO_4 concentrations indicated

PO_4 IN CULTURE SOLUTION	CORN		SORGHUM		TOMATOES	
	Tops	Roots	Tops	Roots	Tops	Roots
p.p.m.	per cent	per cent	per cent	per cent	per cent	per cent
0.05	0.33	0.46	0.32	0.46	0.52	0.80
0.10	0.69	0.68	0.61	0.59	0.56	0.93
0.20	0.89	0.75	0.97	0.85	0.72	1.34
0.50	1.11	0.89	1.24	1.04	1.22	1.65

TABLE 6

PO_4 content of sap from plants grown in culture solutions with phosphate concentrations indicated

PO_4 IN CULTURE SOLUTION	CORN		SORGHUM		TOMATOES	
	Leaves	Stems	Leaves	Stems	Leaves	Stems
p.p.m.	p.p.m.	p.p.m.	p.p.m.	p.p.m.	p.p.m.	p.p.m.
0.05	167	76	183	108	152	86
0.10	185	55	320	68	204	118
0.20	735	68	610	185	270	180
0.50	1,200	196	1,000	430	420	320

As previously indicated, the plant sap was obtained by means of a screw press after the plants had been frozen and permitted to thaw. The sap was then centrifuged until it was perfectly clear. The centrifuging required only about five minutes. The phosphate determinations were made in duplicate by the colorimetric method. Table 6 shows that the PO_4 content of the sap from the leaves and stems increased with increasing concentrations of PO_4 in the culture solutions. The increase was greatest in corn and smallest in tomatoes. The PO_4 concentration in sap from the leaves was higher than that from the stems in all cases. The sap of corn leaves at maximum growth had a much higher PO_4 content than that of sorghum or tomatoes, whereas that from the corn stems had a lower PO_4 content than that from sorghum or tomatoes.

EXPERIMENT 2

This experiment was planned to study in more detail points brought out in experiment 1. Six corn, three sorghum, and three tomato plants were grown for 76 days in each 1,000-liter culture vessel. The culture solutions used had the same composition as those used in experiment 1. Studies were made of PO_4 absorption, rate of growth, total yield of dry matter, and PO_4 content of plant and plant sap. In addition, a study was made of the influence of PO_4 content of the sap on its buffer capacity.

Corn and sorghum were grown in soil cultures at the same time as the plants in the solution culture. A quantity of soil was obtained from plot 2 of the Cullars' Rotation; this soil is very low in phosphate. The soil was placed in 4-gallon earthenware pots. All of the pots were fertilized with muriate of

TABLE 7

*Amount of phosphate absorbed, in weekly periods, from culture solutions of the PO_4 content indicated**

ABSORPTION PERIOD	PO_4 (P. M.) IN CULTURE SOLUTIONS			
	0 05	0 10	0.20	0.50
	mgm.	mgm.	mgm.	mgm.
First week.....	82	100	165	410
Second week.....	16	42	80	190
Third week.....	38	28	38	60
Fourth week.....	27	42	84	370
Fifth week.....	22	66	252	860
Sixth week.....	29	118	504	1,445
Seventh week.....	8	232	812	2,110
Eighth week.....	0	326	704	2,350
Ninth week.....	0	205	725	2,150

* The plants were 7 days old when the absorption study was started.

potash (100 pounds an acre) and sodium nitrate (500 pounds an acre). Half of these soil cultures were fertilized with superphosphate (1,000 pounds an acre) and are designated as high phosphate cultures. The other half of the soil cultures received no phosphatic fertilizer and are designated as low phosphate cultures. These soil cultures were included to compare the growth obtained in soil cultures containing high and low phosphate with the growth in culture solutions.

Rate of PO_4 absorption

The influence of PO_4 concentration of the culture solution on the rate of absorption of PO_4 by plants was studied by determining the amount absorbed each day for nine weeks (table 7).

The amount of PO_4 absorbed during the first week was higher than that

absorbed during the second and third weeks, probably because of the change in the root system of the corn and sorghum plants. The plants were 2 weeks old after they had been in the culture solutions one week. At this time there is a gradual disappearance of the temporary root system and a beginning of the permanent root system.

The PO_4 absorbed from the culture solutions increased with the phosphate concentration of the solutions, but the rate was not directly proportional to the concentration. In most cases the PO_4 absorbed was more than doubled as the PO_4 concentration of the culture solution increased. The plants, however, were larger and had a better developed root system in the culture solutions with high PO_4 concentrations than did those at low PO_4 concentrations. This would afford more absorbing surfaces. The plants grown in the culture solutions at 0.05 p.p.m. PO_4 absorbed very little PO_4 during the seventh week

TABLE 8

The average weekly increase in height of plants grown in culture solutions at PO_4 concentrations indicated

GROWTH PERIOD	CORN				SORGHUM			
	PO_4 (p.p.m.) in culture solutions				PO_4 (p.p.m.) in culture solutions			
	0.05	0.10	0.20	0.50	0.05	0.10	0.20	0.50
weeks	cm.	cm.	cm.	cm.	cm.	cm.	cm.	cm.
2	7.5	7.5	8.1	7.6	8.8	7.7	10.0	10.0
3	19.5	22.3	23.9	24.4	3.2	5.3	5.0	5.0
4	7.0	10.0	14.0	25.0	1.0	2.0	3.0	8.0
5	0.0	13.0	30.0	41.0	0.0	6.0	15.0	18.0
6	1.0	19.0	27.0	36.0	0.0	11.0	20.0	25.0
7	0.0	21.0	52.0	45.0	0.0	12.0	23.0	41.0
8	0.0	20.0	24.0	38.0	0.0	21.0	31.0	35.0
Average....	4.6	17.6	28.5	34.9	0.7	9.5	16.1	22

and none thereafter, because they were practically dead. It is shown that the phosphate concentration of the culture solution has a decided influence on the rate at which PO_4 is absorbed by plants.

Rate of growth and yields

The average height of the plants grown in culture solutions with the various PO_4 concentrations was determined each week, beginning after the plants had been in the culture solutions two weeks. The results for corn and sorghum are presented in table 8. When the corn plants had been in the culture solution two weeks they were fairly uniform in height (table 8). During the third week the difference in rate of growth was small. After this period the rate of growth increased with the increased PO_4 concentration of the nutrient solution. The corn grown at 0.05 p.p.m. PO_4 made no growth after the fourth

week. The rate of growth of the corn plants in solutions at higher PO_4 concentrations increased each week. The diameter of the plants increased with the increased phosphate concentrations of the culture solutions. Plants which were grown at 0.2 and 0.5 p.p.m. PO_4 were stocky, whereas those grown at 0.05 and 0.1 p.p.m. were spindling. The sorghum plants in the various solutions grew at a slower rate than the corn plants, otherwise the rate of growth was influenced by the PO_4 concentration of the culture solution in a similar way.

No measurements were recorded after the plants were 8 weeks old, but the rate of growth increased with the PO_4 concentration of the culture solution until harvest.

The rate of growth was also determined by weighing the plants each week, after allowing the solution to drain from the root system. The results are shown in table 9. There was practically no difference in the rate of corn growth in culture solutions with various phosphate concentrations during the

TABLE 9

Increase in green weight of 3 plants grown in culture solutions at PO_4 concentrations indicated

GROWTH PERIOD	CORN				SORGHUM				TOMATOES			
	PO_4 (p.p.m.) in culture solutions				PO_4 (p.p.m.) in culture solutions				PO_4 (p.p.m.)in culture solutions			
	0.05	0.10	0.20	0.50	0.05	0.10	0.20	0.50	0.05	0.10	0.20	0.50
weeks	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.
2	6.9	4.7	5.1	7.8	3.4	1.2	3.3	0.8	12.2	11.4	15	29
3	12.0	12.3	13.0	11.7	1.0	1.3	2.2	2.5	1.0	3.5	2.1	13
4	14.0	26.2	40.2	91.5	1.5	4.2	3.8	7.5	0.0	3.0	3.5	21
5	1.2	35.5	96.7	261.0	0.3	3.0	7.5	22.2	0.0	10.1	3.0	14
6	7.9	69.3	211.0	546.0	4.8	12.3	34.2	59.0	0.8	26.0	20.4	59
7	4.5	134.0	577.0	1,133.0	0.0	28.0	86.0	150.0	0.0	15.0	39.0	142
8	0.0	174.0	667.0	776.0	0.0	72.0	155.0	166.0	0.0	64.0	44.0	236

second week. But after this period the rate of growth increased markedly with the increased phosphate concentration, as measured by the weight of the plants. Corn grown at 0.05 p.p.m. PO_4 made practically no growth after the fourth week, whereas at the other PO_4 concentrations the plants grew rapidly each week. Sorghum grew much slower than corn, but the PO_4 in the culture solutions affected its growth in the same direction. It may be seen from table 9 that the tomato plants grew more rapidly in the solutions containing 0.5 p.p.m. PO_4 than did those in solutions at 0.1 and 0.2 p.p.m. PO_4 . The average weekly increase at the highest PO_4 concentration was about 80 gm. compared to 20 gm. at 0.1 p.p.m. PO_4 , whereas there was practically no growth at 0.05 p.p.m. PO_4 .

After these plants were harvested, they were divided into three parts; leaves, stems, and roots. It was desirable to obtain a small quantity of sap from the leaves and stems. Therefore, it was necessary to determine the green weights

TABLE 10
Dry weight of plants grown in culture solutions of the phosphate content indicated

PO ₄	CORN—6 PLANTS					SORGHUM—2 PLANTS					TOMATOES—3 PLANTS				
	Stems	Leaves	Tops	Roots	Total	Stems	Leaves	Tops	Roots	Total	Stems	Leaves	Tops	Roots	Total
<i>P. A. M.</i>	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.
0.05	0.5	3.6	4.1	3.1	7.2***	0.3****	0.3*
0.10	78.1	62.5	140.6	45.7	186.3	18.7	9.5	28.2	14.7	42.9	17.0	17.8	34.8	19.1	53.9
0.20	663.9	139.0	802.9	98.6	901.5	48.4	13.0	61.4	18.1	79.5	22.7	30.9	53.6	20.8	74.4
0.50	946.0	179.8	1,125.8	136.8	1,262.6	81.6	17.5	99.1	14.5	113.6	71.9	54.1	126.0	45.8	172.8
High-PO ₄	118.0	86.1	204.1	123.0	327.1	59.0	6.6	65.6	13.3	78.9
Low-PO ₄ } Soil	90.6	84.0	174.6	51.0	225.6	25.8	6.5	32.3	7.2	39.5

* Not determined.

of the plants grown in the various cultures. After a representative sample of the green material for the sap studies was obtained, the remaining green material was dried in an oven for three days at 70°C.; the dry weights were determined and the total dry weights of the plants produced in each culture calculated. The average dry weight of the plants grown in duplicate cultures is given in table 10.

Corn made very little growth in the culture solution containing 0.05 p.p.m. PO_4 ; the dry weight of the tops was only 4.1 gm. The corn tops grown at 0.10 p.p.m. PO_4 made about 35 times as much growth as those grown at 0.05 p.p.m. whereas the roots were about 15 times as heavy as those at the lowest PO_4 concentration. The dry weights of the corn plants increased with the PO_4 concentration of the nutrient solution. A concentration of at least 0.50 p.p.m. PO_4 is required for maximum growth of corn. It is unfortunate that phosphate concentrations higher than 0.5 p.p.m. were not included in this study. The data indicate that high PO_4 concentration in the culture solutions increased the weight of the corn stems to a greater extent than that of the leaves. The top:root ratio increased with the phosphate concentration of the culture solution.

The low- PO_4 soil cultures, containing approximately 0.02 p.p.m. inorganic PO_4 in the soil solution, produced a better growth of corn than did the culture solutions with 0.1 p.p.m. PO_4 . Corn also grew better in the high- PO_4 soil cultures than in culture solutions at 0.1 p.p.m. PO_4 . The PO_4 content of the soil solution was not determined in this experiment, but it was probably not more than 0.1 p.p.m. PO_4 .

The growth of sorghum and tomatoes increased with the phosphate concentration of the culture solution, as shown in table 10. The dry weights of plants grown at 0.05 p.p.m. PO_4 are not recorded because the plants were misplaced after the sap was obtained; they made, however, practically no growth in the culture solutions. The dry weight of sorghum tops was approximately 60 per cent more at 0.5 p.p.m. PO_4 than at 0.2, whereas the increase in the dry weight of tomato tops was 135 per cent. It was shown in experiment 1 that sorghum and tomatoes made about the same growth in culture solutions containing 0.2 and 0.5 p.p.m. PO_4 . This difference may be due to the more favorable growing season. If a factor is limiting growth in some of the cultures and not in others, the differences in growth will be greater with more favorable temperature and light conditions.

PO_4 content of plants and plant sap

The dry tissue of the plants that were grown in culture solutions containing various phosphate concentrations was finely ground and the percentage of PO_4 determined by the method used in experiment 1. The average results of duplicate determinations are shown in table 11.

The data show that the PO_4 content of the plants increases with increasing concentrations of PO_4 in the culture solutions, just as in experiment 1. The

increase was greatest in corn and smallest in sorghum. With all of the ~~the~~ the increase in PO_4 content was greater in the tops than in the roots. The roots of the plants contained a higher percentage of PO_4 than the leaves when grown at 0.05 and 0.1 p.p.m. PO_4 , but the reverse is true of the plants grown at 0.2 and 0.5 p.p.m. PO_4 . Tomatoes had a higher percentage of PO_4 than corn or sorghum. The percentage of PO_4 in the plants grown in experiment 2 is lower than that of those grown in experiment 1.

The sap from the plants grown in culture solutions with different PO_4 concentrations was obtained and clarified; and the inorganic PO_4 content was determined as described in experiment 1. The results are recorded in table 12.

TABLE 11

PO_4 content of dry tissue of plants grown in culture solutions of the phosphate content indicated

PO_4 IN CULTURE SOLUTION	CORN			SORGHUM			TOMATOES		
	Leaves	Stems	Roots	Leaves	Stems	Roots	Leaves	Stems	Roots
	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent
0.05	0.25	0.15	0.44	...	0.11	0.46	0.39	No determinations made	1.17
0.10	0.38	0.27	0.40	0.33	0.29	0.42	0.81		0.85
0.20	0.60	0.43	0.46	0.41	0.35	0.53	0.75		0.96
0.50	0.82	0.62	0.73	0.63	0.58	0.63	1.14		1.34

TABLE 12

PO_4 content of sap from plants grown in culture solutions with phosphate concentrations indicated

PO_4 IN CULTURE SOLUTION	CORN		SORGHUM		TOMATOES	
	Leaves	Stems	Leaves	Stems	Leaves	Stems
	p.p.m.	p.p.m.	p.p.m.	p.p.m.	p.p.m.	p.p.m.
0.05	380	47
0.10	235	55	215	52	210	111
0.20	490	51	385	58	315	161
0.50	1,250	141	581	82	438	500

The phosphate concentration of the culture solutions influenced the PO_4 content of the plant sap. The data show that the sap from the leaves contains a higher concentration of PO_4 than that from the stems except in the case of tomatoes grown at 0.5 p.p.m. PO_4 . Sap from corn plants grown at 0.05 p.p.m. PO_4 contained a rather high PO_4 content, but this may be attributed to the fact that the plants were dried and practically dead when harvested. Sap from corn leaves grown at 0.1 p.p.m. PO_4 contained about 235 p.p.m. PO_4 , whereas the sap from those grown at 0.5 p.p.m. PO_4 contained approximately 1,250 p.p.m.

The sap from sorghum leaves and stems contained less PO_4 than that from corn leaves and stems. No sap could be obtained from sorghum grown in culture solutions at 0.05 p.p.m. PO_4 because the plants were dead when

harvested. An increased PO_4 concentration in the culture solution increased the PO_4 content of the sap from sorghum and tomato plants just as in the case of corn. The sap from tomato leaves had a lower PO_4 content than that from corn and sorghum.

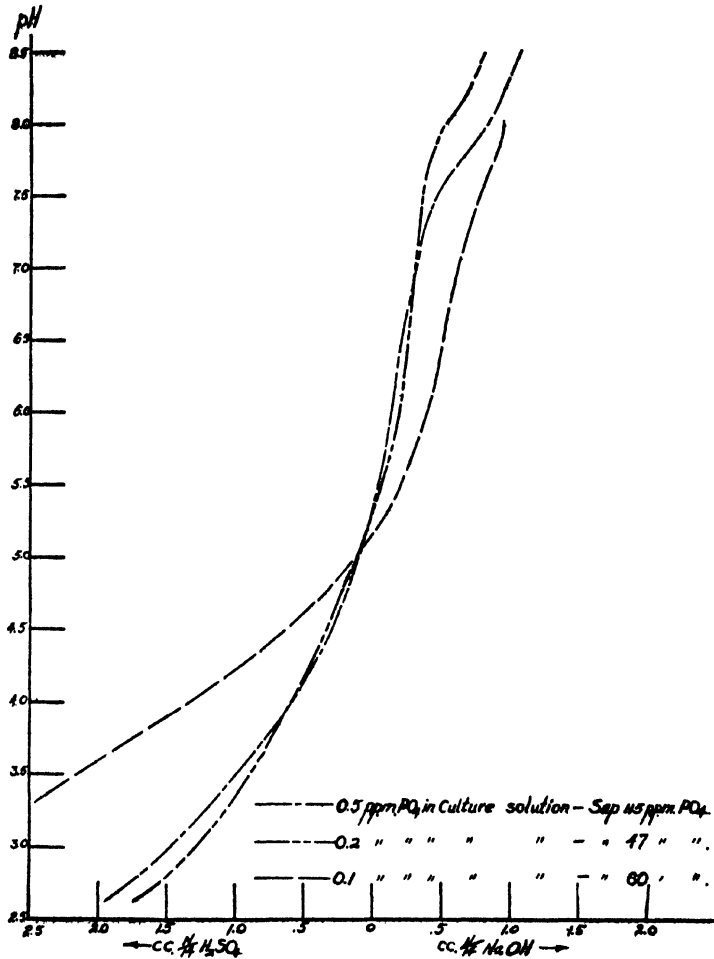


FIG. 1. PHOSPHATE CONTENT AND TITRATION CURVES FOR SAP FROM CORN STEMS GROWN WITH DIFFERENT CONCENTRATIONS OF PHOSPHATE

Buffer capacity of plant sap as related to its PO_4 content

As shown in experiment 1, the PO_4 content of the plant sap was high even in plants that did not make maximum growth. The question arises as to why such concentrations of PO_4 in the sap are inadequate for maximum growth. If

even higher concentrations of PO_4 in the plant sap are necessary for maximum growth, what is the function of the phosphate in the sap? It seemed possible that the phosphate played an important rôle as buffer material in the plant sap and that the high concentrations were essential for properly buffering the sap. Such an assumption seemed to be in accord with the results obtained by Martin (6) on the relation between the PO_4 content and buffer capacity of sap from the leaves and stems of the sunflowers. She found that the buffer capacity of the plant sap was always equal to that which should have been caused by the PO_4 contained in it. She concluded, therefore, that phosphate is the principal buffer in the plant. If her conclusion is correct, the plant sap

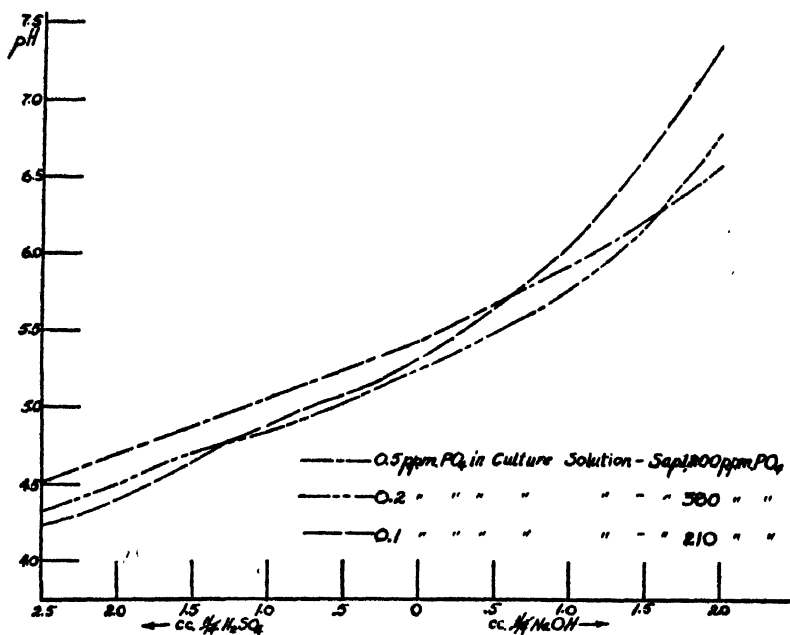


FIG. 2. PHOSPHATE CONTENT AND TITRATION CURVES FOR SAP FROM CORN LEAVES GROWN WITH DIFFERENT CONCENTRATIONS OF PHOSPHATE

containing 1,200 p.p.m. PO_4 should be much better buffered than that containing 200 p.p.m. In order to study this question, the buffer capacity of sap from plants grown in experiment 2 was determined.

In making the study of buffer capacity, the sap was obtained as previously indicated. Its buffer capacity was determined by the change in reaction caused by small additions of $N/14 \text{ H}_2\text{SO}_4$ and NaOH to 5 cc. of the sap. The reaction was determined by means of the quinhydrone electrode.

The titration curves are shown in figures 1 to 4, inclusive. Figure 1 shows the PO_4 content and the titration curves for the sap from corn stems grown in culture solutions with 0.1, 0.2, and 0.5 p.p.m. PO_4 . There is no difference in the buffer capacity of sap which contained 47 p.p.m. and that which con-

tained 115 p.p.m. PO_4 . The sap with 60 p.p.m. PO_4 had a greater buffer capacity than that containing more PO_4 . Figure 2 shows the PO_4 content and the titration curves for the sap from corn leaves grown in culture solutions at various PO_4 concentrations. These curves indicate very little difference in the buffer capacity of sap containing 210, 380, or 1,200 p.p.m. PO_4 . The PO_4 content of the sap from sorghum stems did not affect its buffer capacity as is indicated in figure 3. There is no difference in the buffer capacity of tomato stem sap which contained 500 p.p.m. and that which contained 161 p.p.m. PO_4 (fig. 4). The buffer capacity of the sap which contained 111

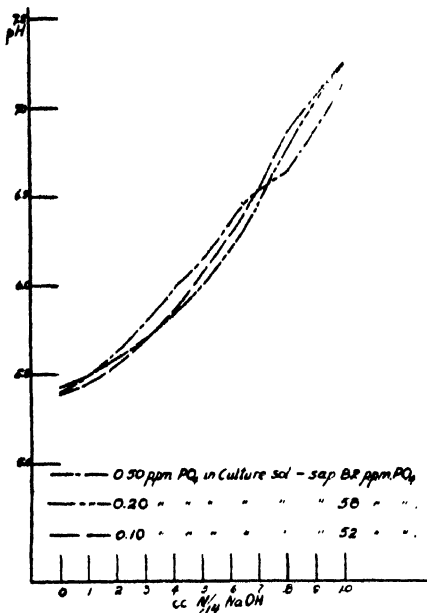


FIG. 3

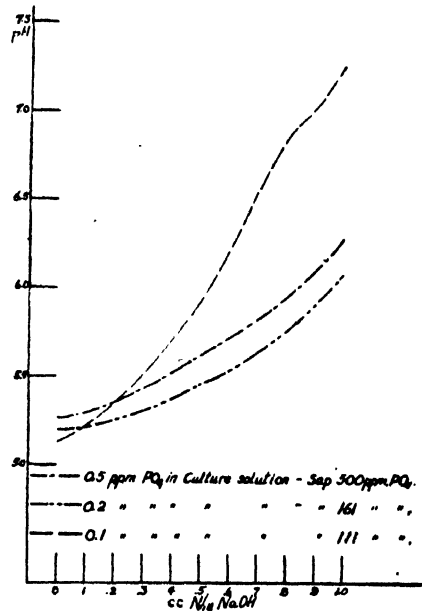


FIG. 4

FIG. 3. PHOSPHATE CONTENT AND TITRATION CURVES FOR SORGHUM STEMS GROWN WITH DIFFERENT CONCENTRATIONS OF PHOSPHATE

FIG. 4. PHOSPHATE CONTENT AND TITRATION CURVES FOR TOMATO STEMS GROWN WITH DIFFERENT CONCENTRATIONS OF PHOSPHATE

p.p.m. PO_4 is much less than that with a higher PO_4 content. In a few cases, there seems to be a rather marked displacement of the curve and perhaps an increased H-ion concentration with low PO_4 content. Probably this is due to the influence of phosphate on metabolic processes concerned with the formation of organic acids, rather than to a lack of phosphate for a buffer material.

The results given in the foregoing indicate very clearly that phosphates play a minor rôle as buffer material in the plant sap and that the apparent necessity of a high phosphate content of plant sap cannot be explained on the basis of its functioning as buffer material.

EXPERIMENT 3

This experiment was planned to study the influence of the reaction of the culture solution on the rate of phosphate absorption by plants. Theron (13) found that the reaction of the culture solution had an appreciable effect on the absorption of Ca, Mg, K, and NO_3 ions. The cations were absorbed more rapidly from an alkaline solution than from an acid solution by barley and

TABLE 13
PO₄ absorbed in 3 days by 16 corn plants at reactions indicated

TRIAL	PO ₄ ABSORBED FROM CULTURE SOLUTIONS OF			
	pH 4	pH 5	pH 6	pH 7.5
	mgm.	mgm.	mgm.	mgm.
1	214	204	138	15
2	164	140	108	44
3	305	266	220	40
Average.....	227	203	155	33

TABLE 14
PO₄ absorbed by 36 wheat plants at reactions indicated

TRIAL	ABSORPTION PERIOD	PO ₄ ABSORBED FROM CULTURE SOLUTIONS OF			
		pH 4	pH 5	pH 6	pH 7.5
	hours	mgm.	mgm.	mgm.	mgm.
1	3	36	36	44	8
2	3	20	18	28	0
3	10	162	208	202	84
4	10	192	202	194	56
5	10	126	110	160	4
6	10	148	146	174	8
7	24	271	263	266	134
8	24	265	274	267	104
9	24	277	282	287	222
10	24	263	269	270	152
Average.....	..	176	180	185	77

cucumbers. The NO_3 ions were absorbed more rapidly from acid solutions whereas the absorption of PO_4 -ions was very irregular. A more detailed study of the influence of the reaction of the culture solution on the absorption of PO_4 was thought desirable.

In an experiment of this kind uniform plants are very essential. Therefore, a large number of seeds were germinated in quartz sand so that a very careful selection of seedlings could be made. Only those having the same appearance, height, and root development were used. Four corn seedlings were placed

in each 100-liter culture vessel. The composition of the culture solution is given in table 1. These plants were grown in the culture solution at the same reaction (pH 6.5) with a very limited amount of phosphate for three weeks. At the end of this period, the plants appeared uniform as to top and root growth, but it was desirable to find out whether or not they were uniform as to PO_4 absorption. The PO_4 content of the culture solutions was adjusted to 1 p.p.m. each day, and the amount of PO_4 absorbed by the plants in each culture was determined for three successive days. In most of the cases the plants in each culture absorbed practically the same amount of phosphate, and those which were not uniform in this respect were discarded. The culture solution were then adjusted to the desired reactions; pH 4, 5, 6, and 7.5 and these reactions were maintained by frequent additions of H_2SO_4 or NH_4OH . There were 4 cultures, or 16 corn plants, and 3 cultures, or 36 wheat plants, at each reaction. The PO_4 content of the culture solutions was again adjusted to 1 p.p.m. each day by the addition of potassium acid phosphate.

The amount of PO_4 absorbed from the culture solutions with the various reactions was determined for several 3-day periods. After each absorption period the plants were placed in 4-gallon pots of tap water for a few days before another absorption period. During the second and subsequent absorption periods the plants were interchanged so that they were never placed in the same solution during any two periods. That is, the plants which were in a solution having a reaction of pH 4 during the first absorption period were subsequently placed in solutions having reactions of pH 5, 6, and 7.5.

The results are shown in tables 13 and 14. The PO_4 absorption by corn (table 13) increased with the acidity of the culture solutions. The PO_4 absorbed was about 30 to 40 per cent more at pH 5 than at 6. In every case the absorption was less at pH 7.5 than at any other reaction. There was no difference in the PO_4 absorption by the wheat plants from the acid culture solutions, as shown in table 14, but the PO_4 absorption was less from the culture solutions having a reaction of pH 7.5.

DISCUSSION

The results obtained in experiment 1 show that a phosphate concentration of at least 0.5 p.p.m. is required for the maximum growth of corn. It also indicates that the maximum growth of tomatoes and sorghum may be obtained at a phosphate concentration of 0.2 p.p.m. The results obtained in experiment 2 are in general agreement with those of experiment 1, except that a concentration of 0.5 p.p.m. PO_4 was required for maximum growth. The cause for this difference is not known, but it may be climatic factors. These results differ considerably from those obtained by Parker and Pierre (11), in that those investigators obtained the maximum growth of corn at a phosphate concentration of 0.25 p.p.m. The cause of this failure to agree with their results is not evident, for the general procedure used in the two investigations was similar, the only known difference being the addition of boron to the culture solution

in the recent experiments. This seems unimportant, however, for Parker and Pierre used tap water and commercial chemically pure salts in preparing their solutions.

When the relation to data on the PO_4 content of the soil solutions is considered, the data obtained in this investigation show rather conclusively the inadequacy of the soil solution, as obtained by displacement, for the growth of plants. Parker (8) has discussed this relation rather fully, so a detailed consideration of it will not be given at this time. In this investigation, however, additional data bearing on the point were obtained by including a few soil cultures for comparison with the solution cultures. As was indicated in experiment 2, corn growing in a soil whose displaced solution contained only 0.02 or 0.03 p.p.m. inorganic PO_4 made better growth than corn in a culture solution containing 0.1 p.p.m. PO_4 .

It is apparent from the foregoing that the root-soil contact is essential for good plant growth in some soils. Comber (2), Parker (8), Hoagland (5), and Truog (14) have discussed this phase of the subject in detail. It will not be discussed here other than to emphasize the fact that it is essential, if we are to account for the absorption of phosphate from many soils of the humid region.

The studies on the composition of the plant and plant sap indicate, as expected, that their phosphate content increases with an increase in the concentration of the culture solution. The sap from the plants which made maximum growth had a very high phosphate content. For maximum growth, why was it necessary for the sap to contain such high PO_4 concentrations? No attempt to answer this question is made, further than to show that the buffer capacity of the sap is not influenced by its phosphate content.

SUMMARY

Two experiments are given in which a study was made of the concentration of PO_4 required for the maximum growth of corn, sorghum, and tomatoes. In addition, the influence of the PO_4 concentration of the culture solution on PO_4 absorption, on rate of growth, on percentage of PO_4 in the dry tissue, on PO_4 content of the plant sap, and on buffer capacity of the sap was studied. The experimental procedure was described in detail. A comparison of the growth of corn and sorghum in soil and solution cultures was made. The influence of the reaction of the culture solution on the rate of PO_4 absorption was studied. The growth of plants in soil and solution cultures was briefly discussed. The results of this investigation may be summarized as follows:

1. Culture solutions of low phosphate concentrations were maintained very well by the use of a large volume of solution for each plant and by the addition of potassium acid phosphate at frequent intervals.
2. The rate of PO_4 absorption was not directly proportional to the PO_4 concentration of the culture solution.
3. The rate of growth increased with increasing phosphate concentrations throughout the growing period.

4. Maximum growth of corn, sorghum, and tomatoes was obtained at 0.5 p.p.m. PO_4 . Growth of corn and sorghum at 0.2 p.p.m. PO_4 was good, the dry weight being about 71 per cent of the maximum. The dry weight of tomatoes at 0.2 p.p.m. was only 42 per cent of the maximum.

5. Plants made a better growth in soil whose displaced solution contained from 0.02 to 0.03 p.p.m. inorganic PO_4 than in culture solutions at 0.1 p.p.m. PO_4 .

6. The phosphate content of plant and plant sap increased with increasing concentrations of PO_4 in the culture solutions. Tomatoes had a higher percentage of PO_4 than corn or sorghum, whereas the sap from corn and sorghum leaves had a PO_4 content higher than that of tomatoes.

7. Phosphate played a minor rôle as a buffer material in the plant sap.

8. Corn and wheat plants absorbed PO_4 more rapidly from acid than from alkaline culture solutions. There was no appreciable difference in the rate of PO_4 absorption from culture solutions having a reaction of pH 4, 5, and 6.

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AN IMPROVED METHOD FOR THE DETERMINATION OF AVAILABLE PHOSPHORIC ACID OF SOILS

SURENDRALAL DAS

Agricultural Research Institute, Pusa, India

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In a previous publication (3) the author has shown that Dyer's citric acid method breaks down as a discriminating agent for evaluating the available phosphoric acid of calcareous soils, and that 1 per cent potassium carbonate solution is capable of differentiating plots of known cropping and manurial history and thus gives an indication of the probable fertility of calcareous soils in their relation to available phosphoric acid. The present investigation was undertaken to determine whether this new method could be applied to other types of soil with equal advantage and thus rendered a universal method for the determination of available phosphoric acid of soils in general.

It is generally conceded that plant-food exists in soils in two forms: one is often referred to as easily soluble, active or available, and consists of that smaller portion which can be utilized by plants for their immediate needs as distinguished from the second form, the larger but less available reserve supply in the soil.

Numerous procedures have been adopted for the estimation of the easily soluble plant-food in soils. The most popular is the weak acid digestion. The mineral acids, hydrochloric or nitric acid, are employed in the United States, whereas citric acid has found favor in Europe and elsewhere.

The reliability of these laboratory methods has been questioned from more than one quarter, and, no doubt, on account of the difficulty of reproducing soil conditions in the laboratory, they have always been justly considered more or less arbitrary. However, it may be safely stated that some of them do give results of a certain value when properly interpreted, as their findings have often been verified in the field; but it is doubtful whether in the present state of our knowledge we can evolve a method on which absolute reliance could be placed.

In this connection the work done at the Texas Agricultural Experiment Station (6, 10, 11) may be of interest. There the solubility of soil minerals has been studied by bringing phosphate or potash minerals into contact with 0.2 *N* HNO_3 —used as a substitute for 1 per cent citric acid solution—in the proportions in which these minerals may occur in the soil and under the conditions of the extraction. Calcium phosphate and precipitated phosphates of iron and aluminium are completely soluble, and vivianite and triplite are nearly so in 0.2 *N* HNO_3 . The aluminum phosphates, e.g., variscite and wavellite, and the basic ferric phosphates are comparatively slightly soluble.

Ferrous phosphate, vivianite, rarely exists in ordinary cultivated soils although it may be found in some soils suffering from lack of aeration. A 0.2 *N* HNO_3 dissolves calcium phosphates completely, but mineral aluminum or basic ferric phosphates to only a slight extent, it differentiates between these two classes of phosphate compounds in the soil. Apatite, phosphate rock, precipitated ferric and aluminum phosphates, vivianite, and triplite are almost equally soluble. Acid phosphate may also be taken as completely soluble. But these compounds can hardly be claimed to possess the same value to plants, nor is a solvent now known which would dissolve phosphoric acid from the phosphates mentioned, in the same proportions as it would be assimilated from them by plants. What we cannot do with known mineral phosphates of known composition and character outside of the soil, we can hardly expect to do with the same phosphates when they are incorporated in the soil, and far less with the unknown phosphates already locked up within the soil.

Soils may, therefore, contain equal quantities of phosphoric acid soluble in 0.2 *N* HNO_3 , and yet liberate unequal quantities for plants, on account of differences in the phosphates present. This consideration must give rise to caution while different types of soil are being compared with one another. Only those soils should be compared which probably contain the same kinds of phosphates. Soils widely dissimilar in origin and character can hardly be compared, unless there is strong evidence that they contain similar phosphates.

Moreover, the nature of the plant which is grown, apart from other factors, must always play an important rôle with regard to the availability of different phosphates present in soils or applied to the same as fertilizers. Truog (9), in a comparison of 10 different kinds of plants grown under greenhouse conditions with acid phosphate, rock phosphate, precipitated calcium phosphates, aluminum phosphate, iron phosphates (both ferrous and ferric), magnesium phosphate, or manganese phosphate as sources of phosphoric acid, found that, contrary to the general belief in the relative unavailability of aluminum and iron phosphates to plants, 9 out of 10 plants made better growth on aluminum phosphate than on calcium phosphates, and 6 made better growth on ferric phosphate. This clearly indicates the inadequacy of chemical solvents in measuring the availability of different phosphates.

Further, many investigators (2,10,11) have observed that the growing plant itself possesses more or less power to feed directly on phosphates and that some plants possess specially marked powers. As a result, no common limiting figure for available phosphoric acid can be suggested, as given by Dyer (4), which will be equally applicable to all types of soil, for the figure varies not only with the character of the soil, but also depends on the kinds of crops grown. Consequently, this figure must be worked out for different soils, and it will vary even in the same soil according to the type of crop grown on it from time to time.

Frazer (5) has dealt with this problem of availability of plant-food in soils from a different stand-point. According to his findings the amount of any

given plant-food which is withdrawn from the soil by the plant depends upon a number of factors, which are classified as follows.

1. The amount of plant-food present at the beginning of the growing season in a form which can be partly or completely absorbed by the plant is termed *chemically available*.
2. Compounds chemically available may be enclosed within the soil particles so as not to be exposed to the action of plant roots. Such compounds are *physically unavailable*. If the encrusting substance is removed, such compounds become chemically available.
3. The amount of plant-food transformed during the growing season into forms of combination which can be absorbed by plants may be measured in terms of *weathering availability*. This factor is certainly of importance with respect to nitrogen. Its importance in the case of potash and phosphoric acid is apparently not so great; but the matter requires study.
4. Plants differ both in their capacity to absorb food and in their need of it. Whatever may be the cause of these differences, there is no doubt that they exist. This factor is designated *physiological availability*.

The character of the soil, its chemical composition, the conditions which prevail during the growth of the plant, the incidence of sunlight, the effective range of temperature, the nature of the microflora present, and the movement of air and water must bring into play several factors affecting the availability of plant-food in soils. Moreover, our knowledge of soil physics and plant physiology needs further development before we can positively assert much in this direction. Physico-chemical studies, however, relating to base exchange and colloidal behavior are likely to give the soil chemist a powerful weapon to combat hitherto baffling problems and to throw new light on many unexplained soil phenomena.

The extraction with 1 per cent potassium carbonate solution has proved useful for the estimation of available phosphoric acid of calcareous soils (3). The underlying principle of its action on calcareous soils is twofold: a reaction takes place with any dicalcic or such other phosphates present in these soils, with the production of insoluble tricalcic or other phosphates and of soluble potassium phosphate; and, further, phosphorus in organic combination in humus is also dissolved.

Dyer's 1 per cent citric acid solution has been shown to be unsuitable for calcareous soils (3), and therefore the results obtained with other types of soil by this method cannot be compared with those of calcareous soils. On the other hand, if the extraction with 1 per cent potassium carbonate solution which has been found useful for calcareous soils, proves equally effective for other types of soil too, then it can be recognized as a more or less universal method for the estimation of available phosphoric acid of soils in general.

In order to test this point as well as to compare the two methods, many typical soils from different parts of India were collected for examination, the cropping and manurial history of these soils being accurately known. In a few cases the total phosphoric acid of soils was also determined with a view to finding out whether it had any significant relationship to available phosphoric acid. The method of extraction with 1 per cent potassium carbonate solution has already been described (3) and is practically the same as Dyer's

TABLE 1

A comparison of total and available phosphoric acid by citric acid and potassium carbonate methods in acid soils of known cultural history

DESCRIPTION OF PLOTS	CROPS GROWN	PER CENT TOTAL P_2O_5 (T)	PER CENT AVAILABLE P_2O_5		$\frac{K}{T}$	$\frac{C}{T}$
			By citric acid (C)	By K_2CO_3 (K)		
Tocklai Indian Tea Association, Assam						
Borbhetta-Kharikatia Plots:						
No manure.....	Tea	0.0352	0.0023	0.0080	0.228	0.066
Superphosphate.....	Tea	0.0414	0.0051	0.0112	0.270	0.124
Basic slag.....	Tea	0.0454	0.0057	0.0105	0.230	0.126
Limestone.....	Tea	0.0406	0.0019	0.0064	0.157	0.046
Limestone + K_2SO_4	Tea	0.0432	0.0053	0.0131	0.304	0.123
Limestone + superphosphate.....	Tea	0.0436	0.0050	0.0103	0.237	0.114
K_2SO_4	Tea	0.0317	0.0020	0.0073	0.231	0.063
K_2SO_4 + superphosphate.....	Tea	0.0443	0.0049	0.0129	0.292	0.112
Borbhetta-Betjan Plots:						
No manure.....	Tea	0.0413	0.0027	0.0099	0.240	0.066
Manured.....	Tea	0.0450	0.0041	0.0126	0.281	0.092
Borbhetta-Matelli Plots:						
Bonedust.....	Tea	0.0434	0.0039	0.0079	0.183	0.090
Bonedust + fish guano.....	Tea	0.0429	0.0042	0.0091	0.263	0.098
Bonedust + nitrolim.....	Tea	0.0484	0.0034	0.0065	0.134	0.071
Bonedust.....	Tea	0.0405	0.0045	0.0107	0.263	0.110
Bonedust + $NaNO_3$	Tea	0.0407	0.0039	0.0080	0.197	0.096
Bonedust + $(NH_4)_2SO_4$	Tea	0.0421	0.0040	0.0080	0.208	0.094
Bonedust + mustard cake.....	Tea	0.0431	0.0041	0.0087	0.201	0.096
Block E, Jorhat Government Farm, Assam						
Non-phosphated.....	Sugar cane (<i>S. Mauritius</i>)	0.0034	0.0083
Phosphated.....	Sugar cane (<i>S. Mauritius</i>)	0.0043	0.0093
Non-phosphated.....	Sugarcane, D. 74	0.0038	0.0090
Phosphated.....	Sugarcane, D. 74	0.0066	0.0096
Block D, Jorhat Government Farm, Assam						
Non-phosphated.....	Sugarcane, D. 74	0.0019	0.0079
Phosphated.....	Sugarcane, D. 74	0.0017	0.0113
Block E, Karimganj Government Farm, Assam						
Control.....	Paddy	0.0006	0.0062
Bonemeal.....	Paddy	0.0021	0.0074

TABLE 1—*Concluded*

DESCRIPTION OF PLOTS	CROPS GROWN	PER CENT TOTAL P ₂ O ₅ (T)	PER CENT AVAILABLE P ₂ O ₅		$\frac{K}{T}$	$\frac{C}{T}$
			By citric acid (C)	By K ₂ CO ₃ (K)		
<i>Hmwabi Experimental Station, Burma</i>						
Bad, unmanured.....	Paddy	0.0339	0.0021	0.0025	0.074	0.061
Good, unmanured.....	Paddy	0.1109	0.0444	0.0260	0.235	0.400
No manure.....	Paddy	0.0409	0.0032	0.0037	0.090	0.078
Bonemeal.....	Paddy	0.0520	0.0097	0.0060	0.116	0.186
<i>Cinchona Camp, via Margui, South Burma</i>						
Bad soil from ridge.....	Cinchona	0.0350	0.0015	0.0046	0.131	0.043
Good soil from slope.....	Cinchona	0.1720	0.0090	0.0079	0.046	0.052

citric acid method except for certain details. For easy reference, however, it is briefly stated in the following.

One-hundred grams of air-dry soil passing 2-mm. mesh are shaken with 1 liter of 1 per cent potassium carbonate solution for 24 hours in a mechanical shaker. The extract, containing some potassium carbonate in the free state, is separated by suction and neutralized almost wholly with nitric acid and then with a little hydrochloric acid; as a result a considerable amount of potassium nitrate is produced in the solution, which helps the granular precipitation of ammonium-phosphomolybdate in the ammonium molybdate method of estimating phosphoric acid.

Soils collected may be broadly divided into three groups; namely,

1. *Acid soils*, in which are also included *humus* and *laterite* soils.
2. *Alkali soils*, which also include *calcareous* soils.
3. *Non-calcareous soils*, including various typical soils not belonging to 1 and 2.

ACID SOILS, INCLUDING HUMUS AND LATERITE SOILS

Acid soils were obtained from the Government Farms of Jorhat and Karimganj, the Tocklai Experimental Station of the Indian Tea Association, Assam, and also from Burma. The soils were collected from plots whose cropping and manurial history was known. The pH of these soils varies from 3.0 to 6.0. The results obtained are set forth in table 1.

It will be noticed that the analytical figures for phosphoric acid obtained with 1 per cent potassium carbonate extraction serve to differentiate manured from unmanured plots, and compare well with those given by Dyer's citric acid method. In some cases total phosphoric acid was determined in order to see whether it had any relationship with the values of available phosphoric acid. The ratio of K_2CO_3 -soluble to total phosphoric acid varies from 0.13 to 0.30 in the case of acid soils of Tocklai Indian Tea Association, Assam,

TABLE 2

Comparison of total and available phosphoric acid by citric acid and potassium carbonate methods in laterite soils of known cultural history

DESCRIPTION OF PLOTS	CROPS GROWN	PER CENT TOTAL P_2O_5 (T)	PER CENT AVAILABLE P_2O_5		$\frac{K}{T}$	$\frac{C}{T}$
			By citric acid (C)	By K_2CO_3 (K)		
East Suti Plots, Dacca						
No manure.....	Paddy and maize	0.0013	0.0079
Bonemeal.....	Paddy and maize	0.0017	0.0097
Basu's Experimental Plots, Dacca						
Bonemeal.....	Paddy	0.0020	0.0081
Bonemeal + lime.....	Paddy	0.0015	0.0063
North Hazi Plots, Blocks B and C, Dacca						
No manure.....	Mustard, paddy, and matikalai (<i>Phaseolus mungo</i>)	0.0618	0.0021	0.0075	0.121	0.034
Bonemeal.....	Mustard, paddy, and matikalai (<i>Phaseolus mungo</i> ;	0.0854	0.0067	0.0099	0.115	0.078
Lime.....	Mustard, paddy, and matikalai (<i>Phaseolus mungo</i>)	0.0675	0.0043	0.0065	0.096	0.064
Lime + bonemeal.....	Mustard, paddy, and matikalai (<i>Phaseolus mungo</i>)	0.0827	0.0127	0.0081	0.098	0.154
Cowdung.....	Mustard, paddy, and matikalai (<i>Phaseolus mungo</i>)	0.0639	0.0025	0.0081	0.127	0.039
Cowdung + bonemeal.....	Mustard, paddy, and matikalai (<i>Phaseolus mungo</i>)	0.0737	0.0089	0.0116	0.157	0.121
Cowdung + lime.....	Mustard, paddy, and matikalai (<i>Phaseolus mungo</i>)	0.0596	0.0027	0.0065	0.109	0.045
Cowdung + lime + bonemeal.....	Mustard, paddy, and matikalai (<i>Phaseolus mungo</i>)	0.0891	0.0154	0.0100	0.112	0.173

TABLE 2—Concluded

DESCRIPTION OF PLOTS	CROPS GROWN	PER CENT TOTAL P_2O_5 (T)	PER CENT AVAILABLE, P_2O_5		$\frac{K}{T}$	$\frac{C}{T}$
			By citric acid (C)	By K_2CO_3 (K)		
North Hazi Plots, Blocks A and D, Dacca						
Green manure.....	Mustard, paddy, and matikalai (<i>Phaseolus mungo</i>)	0.0609	0.0018	0.0072	0.118	0.030
Green manure + bonemeal.....	Mustard, paddy, and matikalai (<i>Phaseolus mungo</i>)	0.0722	0.0042	0.0090	0.125	0.058
Green manure + cowdung.....	Mustard, paddy, and matikalai (<i>Phaseolus mungo</i>)	0.0021	0.0096
Green manure + cowdung + lime..	Mustard, paddy, and matikalai (<i>Phaseolus mungo</i>)	0.0007	0.0075
Green manure + lime.....	Mustard, paddy, and matikalai (<i>Phaseolus mungo</i>)	0.0489	nil	0.0054	0.110
Green manure + lime + bonemeal.	Mustard, paddy, and matikalai (<i>Phaseolus mungo</i>)	0.0833	0.0079	0.0071	0.085	0.095
Green manure + bonemeal + cowdung.....	Mustard, paddy, and matikalai (<i>Phaseolus mungo</i>)		0.0059	0.0128
Green manure + bonemeal + cowdung + lime.....	Mustard, paddy, and matikalai (<i>Phaseolus mungo</i>)	0.0128	0.0100

whereas the corresponding ratio in the case of citric-soluble phosphoric acid ranges between 0.04 and 0.12. The variation of these ratios is still greater in Burma acid soils. It will be evident that the greater fertility of the plots examined with respect to available phosphoric acid is usually accompanied by proportionally increasing ratios by both the methods.

Similarly, Lemmermann (7) and Andre and Copaux (1), from a determina-

tion of the ratio of citric-soluble to total phosphoric acid of soils of known agricultural history, concluded that the greater the ratio between citric-soluble and total phosphate, the smaller the return which follows the application of soluble phosphate, and that it is not so much the absolute dose of phosphoric acid that matters with regard to the factor of fertility as the size of the preceding ratio. Vanstone (12) also compares the value of determinations of this ratio with the information given by the Dyer's method.

Next, laterite soils collected from Dacca Central Farm were examined for available phosphoric acid. These soils are acid in reaction, the pH varying from 4.0 to 6.0. The results are given in table 2.

TABLE 3
Comparison of total and available phosphoric acid by citric acid and potassium carbonate methods in humus soils of known cultural history

DESCRIPTION OF PLOTS	CROPS GROWN	PER CENT TOTAL P_2O_5 (T)	PER CENT AVAILABLE P_2O_5		$\frac{K}{T}$	$\frac{C}{T}$
			By citric acid (C)	By K_2CO_3 (K)		
<i>Hill soils, Simla</i>						
Polo ground.....	Grassland	0.286	0.0622	0.0348	0.122	0.218
Race course.....	Grassland	0.360	0.1208	0.0424	0.118	0.336
<i>Somerford Orchard, Ramgarh, District Nainital, U. P.</i>						
Soil no. 2.....	Fruit trees	0.136	0.0131	0.0106	0.078	0.097
Soil no. 3.....	Fruit trees	0.235	0.0409	0.0095	0.040	0.174
<i>Hill soils, Cloud End, Mussoorie</i>						
Soil no. A.....	Fruit trees	0.159	0.035	0.0104	0.065	0.220
Soil no. C.....	Fruit trees	0.377	0.117	0.0348	0.092	0.470
Soil no. D.....	Fruit trees	0.327	0.048	0.0216	0.066	0.147
Soil no. E.....	Fruit trees	0.353	0.100	0.0322	0.091	0.283
Soil no. F.....	Fruit trees	0.195	0.015	0.0148	0.076	0.077

The same remarks apply here as in the case of acid soils reported in table 1.

Hill soils are generally very rich in humus and available plant-food, but poor in lime content. They are acid in reaction. Some such soils of known agricultural history were obtained from Simla, Mussoorie, and Ramgarh for examination. The results are given in table 3.

Here, too, the same remarks apply as in the case of other acid soils of this group.

ALKALI SOILS INCLUDING CALCAREOUS SOILS

Results of alkali soils obtained from Sukkur Agricultural Station, Sindh, are given in table 4. Good soils represent those which are being reclaimed

under the Sukkur Barrage Scheme recently brought into being, and are more fertile than bad soils which have as yet been little reclaimed. The determination of total phosphoric acid was omitted in soils of this group.

It will be noticed that the citric acid method breaks down altogether as a criterion for distinguishing good soils from bad ones, and, as a matter of fact, carries an erroneous impression with regard to the fertility of these soils. On the other hand, the values obtained with potassium carbonate solution are borne out by the cultural history of the plots under examination.

In this group are also included calcareous soils obtained from the Bombay Presidency, Central Provinces, and Berars. The results are set forth in table 5.

In the case of soils from Manjri Dry Farming Station, Bombay, the citric acid extraction did not yield any phosphoric acid which could be estimated by ordinary methods of analysis. The figures obtained with potassium car-

TABLE 4

Comparison of available phosphoric acid by citric acid and potassium carbonate methods in alkali soils of known cultural history

DESCRIPTION OF PLOTS	CROPS GROWN	PER CENT AVAILABLE P_2O_5	
		By citric acid	By K_2CO_3
Bad soil.....	Sorghum, berseem and wheat	0.0198	0.0043
Good soil.....	Sorghum, berseem and wheat	0.0031	0.0071
Bad soil.....	Sorghum	0.0326	0.0036
Good soil.....	Sorghum	0.0206	0.0069

bonate solution, though not very conclusive, yet give some indication of the fertility of the lands. In the case of soils of Poona Agricultural College Farm, black soils of Nagpur College Farm, and black cotton soils of Akola Government Farm the potassium carbonate method gives values of available phosphoric acid which are clearly corroborated by field tests, whereas the results with the citric acid process are rather erratic.

Thus it will be noticed that in practically all cases examined, the method of extracting with 1 per cent potassium carbonate solution has differentiated between manured and unmanured plots and also between plots treated with phosphatic fertilizers and those treated with fertilizers containing no phosphates, whereas the citric acid method yields uneven and even misleading values in most cases.

In the present instance, as the total amount of phosphoric acid of these soils was not determined, the ratios of potassium carbonate-soluble or citric-soluble to total phosphate could not be compared. The results of calcareous soils detailed in a previous publication by the author (3), where total phosphoric

acid was determined, show that the greater fertility of soils under examination with respect to their available phosphoric acid is generally accompanied by proportionally bigger ratios obtained with the potassium carbonate method.

TABLE 5

Comparison of available phosphoric acid by citric acid and potassium carbonate methods in calcareous soils of known cultural history

DESCRIPTION OF PLOTS	CROPS GROWN	PER CENT AVAILABLE P_2O_5	
		By citric acid	By K_2CO_3
<i>Manjri Dry Farming Station, Bombay</i>			
Plot no. 2, control.....	Bajri (<i>Pennisetum typhoideum</i>)	nil	0.0013
Plot no. 6, farmyard manure.....	Bajri (<i>Pennisetum typhoideum</i>)	nil	0.0014
Plot no. 7, green manure.....	Bajri (<i>Pennisetum typhoideum</i>)	nil	0.0012
Control, shallow.....	Bajri (<i>Pennisetum typhoideum</i>)	nil	0.0011
<i>Poona Agricultural College Farm, Bombay</i>			
No manure.....	Horse gram	0.0024	0.0023
Plot no. 6A, formyard manure.....	Horse gram	0.0024	0.0036
Plot no. 6B and 5B, farmyard manure.....	Horse gram	0.0011	0.0034
Plot no. 23A, farmyard manure.....	Horse gram	0.0286	0.0050
Plot no. 21A and 22A, farmyard manure.....	Horse gram	0.0406	0.0065
<i>Black soil, Nagpur College Farm, C. P.</i>			
Dung + urine.....	Sorghum and gram*	0.0009	0.0012
Dry excreta.....	Sorghum and gram*	0.0002	0.0011
Urine.....	Sorghum and gram*	0.0010	0.0008
No manure.....	Sorghum and gram*	0.0016	0.0005
Scarified.....	Wheat†	0.0109	0.0023
Country ploughed and scarified.....	Wheat†	0.0076	0.0024
Inversion ploughed and scarified.....	Wheat†	0.0150	0.0032
<i>Black cotton soils, Akola Government Farm, Berars</i>			
Castor cake + super.....	Cotton*	0.0041	0.0011
Castor cake + super + lime.....	Cotton*	0.0006	0.0009
Castor cake + super + lime.....	Cotton*	0.0006	0.0006

* Yields in descending order.

† Yields in ascending order.

Such relationship would not obviously hold in the case of the Dyer's method, which gives rather erratic results in these soils.

NON-CALCAREOUS SOILS

These soils represent approximately neutral soils, being neither too acid nor too alkaline in reaction. The results are set forth in table 6. In a few cases total phosphoric acid was also determined.

It will be noticed that in practically all cases examined both the methods of extraction have differentiated between manured and unmanured plots, and also between plots treated with phosphatic fertilizers and those treated with other fertilizers containing no phosphates. It is only in the two groups of acid and non-calcareous soils that both methods agree closely, whereas in the case of calcareous and alkali soils the citric acid method gives erratic results. On the other hand, the method of extracting with 1 per cent potassium carbonate solvent gives, in a uniform manner and in all types of soil, values for available phosphoric acid which are significantly related to the cultural history of the plots, and are therefore comparable with regard to the probable fertility of soils in their relation to available phosphoric acid. This possibility is rather remote in the case of the citric acid process, for reasons already detailed above. The same fact will obviously hold true in the case of any of its substitutes dependent upon acid digestion.

Further, the potassium carbonate solvent is capable of dissolving phosphorus in organic combination in humus, which citric acid solution fails to do. It is well known that phosphorus in organic combination generally remains in a colloidal and highly dispersed state in the soil solution, and, as such, is believed to be more efficacious and easily available to plants than inorganic phosphates in soils (8). Thus it may perhaps be maintained that potassium carbonate solution, which extracts phosphorus in both organic and inorganic combinations in soils, is a very suitable solvent for the estimation of soil phosphates which will be available for plant nutrition. Consequently, the potassium carbonate method is capable of measuring the probable fertility of all types of soil with respect to available phosphoric acid, and, as such, can be recommended as of universal application for this purpose. Besides, citric acid solution extracts, in the majority of cases, especially in calcareous soils, very small amounts of phosphoric acid, giving rise to great manipulative difficulties in their estimation. Thus the potassium carbonate method is a decided improvement on the existing citric acid method.

SUMMARY AND GENERAL CONCLUSIONS

A large number of typical soils of known cropping and manurial history, consisting of acid, laterite, humus, alkali, calcareous, and non-calcareous ones, were collected from different parts of India for a comparison of the new potas-

TABLE 6'
Comparison of total and available phosphoric acid by citric acid and potassium carbonate methods in non-calcareous soils of known cultural history

DESCRIPTION OF PLOTS	CROPS GROWN	PER CENT TOTAL P ₂ O ₅ (T)	PER CENT AVAILABLE P ₂ O ₅		K T	C T
			By citric acid (C)	By K ₂ CO ₃ (K)		
Coimbatore Permanent Experimental Plots, Madras						
No manure.....	Sorghum, ragi (<i>Eleusine coracana</i>), and wheat	0.0428	0.0118	0.0010	0.022	0.276
P.....	Sorghum, ragi (<i>Eleusine coracana</i>), and wheat	0.0790	0.0600	0.0016	0.020	0.759
N.....	Sorghum, ragi (<i>Eleusine coracana</i>), and wheat	0.0516	0.0138	0.0010	0.025	0.267
N + P.....	Sorghum, ragi (<i>Eleusine coracana</i>), and wheat	0.1515	0.0917	0.0026	0.017	0.606
N + K.....	Sorghum, ragi (<i>Eleusine coracana</i>), and wheat	0.0845	0.0455	0.0016	0.019	0.538
N + K + P.....	Sorghum, ragi (<i>Eleusine coracana</i>), and wheat	0.1421	0.0847	0.0026	0.018	0.596
Cattle manure.....	Sorghum, ragi (<i>Eleusine coracana</i>), and wheat	0.0463	0.0139	0.0016	0.034	0.295
Cattle manure, residual, manuring discontinued since 1916..	Sorghum, ragi (<i>Eleusine coracana</i>), and wheat	0.0308	0.0131	0.0017	0.055	0.427
Nandyal Agricultural Station, Madras						
No manure.....	Sorghum and cotton	0.0034	0.0005
Cattle manure.....	Sorghum and cotton	0.0300	0.0014
No manure.....	Sorghum and cotton	0.0065	0.0005
Cattle manure.....	Sorghum and cotton	0.0111	0.0010

<i>Koilpatti Agricultural Station, Madras</i>						
No manure.....	Cumbu (<i>Pennisetum typhoides</i>)	0.0074	0.0001
Superphosphate.....	Cumbu (<i>Pennisetum typhoides</i>)	0.0096	0.0010
Superphosphate + cyanamide.....	Cumbu (<i>Pennisetum typhoides</i>)	0.0097	0.0010
<i>Dharwar Farm, Bombay</i>						
Non-phosphated.....	Cotton and tobacco	0.0001	0.0011
Phosphated.....	Cotton and tobacco	0.0006	0.0012
<i>Gokak Canal Farm, Bombay</i>						
Non-phosphated.....	Sugarcane	0.0258	0.0039
Phosphated.....	Sugarcane	0.0397	0.0058
<i>Dohad Farm, Panch Mahals, District Guzerat, Bombay</i>						
Plot no. 70, good plot.....	Maize and gram	0.0120	0.0020
Plot no. 71, supered in 1914.....	Maize and gram	0.0064	0.0016
<i>Indore soil, Central India</i>						
Light cotton soil, unmanured.....	Cotton	0.0018	0.0001
Light cotton soil, fertile.....	Cotton	0.0070	0.0028
Deep cotton soil, unmanured.....	Cotton	0.0015	0.0009
Deep cotton soil, manured.....	Cotton	0.0085	0.0026
<i>Etwah Government Farm, U. P.</i>						
No manure.....	Gram, peas, and wheat	0.0518	0.0083
Castor cake.....	Gram, peas, and wheat	0.0379	0.0119
Farmyard manure.....	Gram, peas, and wheat	0.0233	0.0044

TABLE 6—*Concluded*

DESCRIPTION OF PLOTS	CROPS GROWN	PER CENT TOTAL P ₂ O ₅ (T)	PER CENT AVAILABLE P ₂ O ₅		$\frac{K}{T}$	$\frac{C}{T}$
			By citric acid (C)	By K ₂ CO ₃ (K)		
Akara Government Farm, District Banda, U. P.						
Kabar soil, no manure	Wheat and paddy	0 0941	0 0274
Rakar soil, no manure ..	Gram and sorghum	0 0115	0 0014
Mar soil, no manure	Wheat and gram	0 0164	0 0052
Parwa soil, green manure	Wheat and sorghum	0 0369	0 0147
Lyallpur Agricultural College Farm, Punjab						
Control	Cotton, maize, and senji fodder (<i>Melilotus parviflora</i>)	0 0831	0 0033
Bonemeal	Cotton, maize, and senji fodder (<i>Melilotus parviflora</i>)	0 0911	0 0038
Gurdaspur Agricultural Station, Punjab						
Field C/6, plot no. 3, control	Wheat	0 0113	0 0041
Field C/6, plot no. 2, super	Wheat	0 0175	0 0062
Field C/6, plot no. 6, complete manure.....	Wheat	0 0210	0 0066
Field A/10, plot no. 11, control	Striped sugarcane	0 0246	0 0049
Field A/10, plot no. 16, super.	Striped sugarcane	0 0311	0 0081
Field A/10, plot no. 10, complete manure.	Striped sugarcane	0 0286	0 0069
Field A/1a, plot no. 11, control	Striped sugarcane	0 0150	0 0059
Field A/1a, plot no. 16, super.	Striped sugarcane	0 0277	0 0117
Field A/1a, plot no. 10, complete manure.	Striped sugarcane	0 0165	0 0075

<i>Cuttack Farm, Orissa</i>					
Plot A, best land.....	Paddy	0.0051	0.0074
Plot B, medium land.....	Paddy	0.0036	0.0068
Plot C, poor land.....	Paddy	0.0009	0.0036
<i>Government Farm, Adhartal, Jubbulpore, C. P.</i>					
Unmanured.....	0.0013	0.0021
Sann-hemp.....	0.0010	0.0024
Sann-hemp + superphosphate.....	0.0012	0.0026
<i>Sabour Government Farm, South Bhagalpur, Bihar</i>					
Poor land, unmanured.....	Gram and linseed	0.0191	0.0052
Fertile land, unmanured.....	Gram and linseed	0.2822	0.0172
Ammonium sulphate.....	Sugarcane	0.1305	0.0171
<i>Kharhara Farm, South Bhagalpur, Bihar</i>					
Unmanured.....	Paddy	0.0008	0.0019
Ammonium sulphate.....	Paddy	0.0016	0.0023
<i>Nawada Government Farm, District Gaya, Bihar</i>					
I. Unmanured, poor crop.....	Gram	0.0004	0.0005
II. Radio-phos (apatite).....	Yield better than I	0.0006	0.0008
III. Super.....	Yield better than I and II	0.0012	0.0012
Residual effect of super.....	Yield better than I and II	0.0011	0.0028

sium carbonate method with Dyer's citric acid method for the estimation of available phosphoric acid. The following conclusions are summarized from the results obtained:

1. The results obtained showed that the potassium carbonate method is equally applicable to all types of soil, whereas the citric acid method breaks down as a discriminating agent for evaluating the available phosphoric acid of alkali and calcareous soils.

2. Besides the obvious advantage of a more general application, the potassium carbonate method possesses several other points in its favour and should therefore replace the existing Dyer's method or any of its substitutes dependent upon acid digestion for estimating the available phosphoric acid of soils.

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NITROGEN FIXATION IN FIELD SOIL UNDER DIFFERENT CONDITIONS OF CROPPING AND SOIL TREATMENT

LEON A. BRADLEY AND JAMES E. FULLER

Massachusetts Agricultural Experiment Station

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The importance of investigating the relationship of bacterial activities in the soil to crop production was emphasized by Brown (2). Perhaps it is equally important to know what influence the crop may have on the bacteria, in order that the complete cycle may be understood. Since it is recognized that certain bacteria, such as nitrifying, nitrogen-fixing, and cellulose-decomposing species, are necessary aids to soil fertility, the complete understanding of such a cycle might make it possible to utilize these microscopic agents more effectively.

The following are a few representative illustrations from the literature dealing with the effects of crops on the microscopic soil flora:

Hiltner (5) stated that growing plant roots stimulate the activities of free-living nitrogen-fixing bacteria. Heinze (4) found that *Azotobacter* appears to be more widely distributed in cultivated than in virgin soil.

Greaves (3) reported the analyses of hundreds of samples of cultivated and virgin soils in Utah and concluded that virgin soils have a low nitrogen-fixing power as compared with cultivated soils. Brown (2) investigated the effect of crop rotation over a few seasons. His results indicated that a rotation of corn and clover resulted in more active nitrogen-fixation than did corn alone. A rotation of corn and cowpeas had little effect, whereas the rotation of corn with rye apparently depressed nitrogen fixation.

Starkey (14) investigated some influences of the development of higher plants upon the microorganisms in the soil. His article is cited especially because it contains a considerable bibliography on the subject.

EXPERIMENTAL

The present investigation is closely connected with a field experiment which has been in progress for some years at the Massachusetts Agricultural Experiment Station on the effects of different crop rotations and fertilizer treatments on crop production. Some of the results of these field experiments have been published by Ruprecht (10), by Ruprecht and Morse (11, 12), and by Morse (6, 7, 8, 9).

The field, designated as field A, is divided transversely from east to west into six strips, each 2 rods wide, which are numbered 5 to 10 inclusive. The original field included four additional strips, 1 to 4 inclusive, but the construction of a new building a few years ago made their abandonment necessary. Strip 10 is across the north end of the field. The field is further divided longitudinally from north to south into four strips each 2 rods wide.

The first longitudinal strip (beginning on the west side of the field) and the third are planted certain years with a legume crop such as soybeans or clover, and the second and fourth are planted with a non-legume such as millet. On alternate years, the whole field is planted with a non-legume crop, corn being used frequently. Thus, the west and east halves of the field are exact duplicates so far as crops are concerned.

The longitudinal strips are designated in this report by the letters *LW* (legume west), *NLW* (non-legume west), *LE* (legume east), and *NLE* (non-legume east). The transverse and longitudinal divisions of the field result in 24 plots each 2 rods square, each of which is designated by the letter of the longitudinal and the number of the transverse strips, as 10 NLW and 6 LE.

TABLE 1
The arrangement and fertilizer treatment of field A
North

	LEGUMES WEST	NON-LEGUMES WEST	LEGUMES EAST	NON-LEGUMES EAST
10		Dry ground fish		
9		No nitrogen		
8		Ammonium sulfate		
7		No nitrogen		
6		Ammonium sulfate until 1922 No nitrogen since		
5		Sodium nitrate		

The field has been used for a continuous experiment on the effects on crops of different plant nutrients, including nitrogen, potash, phosphorus, and lime compounds. Strip 5 has been treated annually with sodium nitrate, strip 8 with ammonium sulfate. Strip 10 was formerly treated with dried blood, and more recently with dried ground fish. Strip 6 was formerly treated with ammonium sulfate but has had no nitrogen since 1922. Strips 7 and 9 have not received any kind of nitrogenous fertilizer since the experiment was begun in 1882. The whole field is treated annually with uniform quantities of potash and phosphorus. The lime treatment is shown in table 4. The treatment of the field and its influence on crops will be found in the articles by Morse (6, 7, 8, 9) and by Ruprecht and Morse (11, 12). The present experiment has been carried out along similar lines since 1923.

Table 1 shows the arrangement of the field and the nitrogen treatment. As a result of the fertilizer treatment and the system of cropping, the 24 individual plots fall into the following groups: those planted with legumes and receiving

nitrogen, those planted with legumes but receiving no nitrogen, those planted with non-legumes and receiving nitrogen, and those planted with non-legumes and receiving no nitrogen.

At the end of each season, the crops are weighed and analyzed for nitrogen content; nitrogen determinations are also made on the soil from time to time. The department of chemistry of the Massachusetts Agricultural Experiment Station has had charge of the experiments outlined in the foregoing and some of the analyses have been made by the department of feed control.

The outstanding results of the crop and soil analyses have been as follows: 1. More nitrogen has been taken off the field with the crops than has been added as fertilizer. 2. Plots which have not received nitrogen but have been planted with legumes have produced as good crops as any of the others. 3. When crops of Japanese millet have been grown on the field, and in some seasons together with corn crops, the yield has been uniformly good over the whole field, including plots which have had no nitrogen added and no legume crops grown on them.

It is the purpose of this investigation to determine what effect the crop rotation and fertilizer treatment may have on nitrogen-fixation, and also whether the nitrogen-fixation is sufficiently active to account for the residual nitrogen observed in parts of the field which have had neither legume crops or fertilizer treatment.

The experimental work has been conducted according to the following four plans:

I. A comparative study of the bacterial flora of the plot soils in field A by quantitative plating on different media and the subsequent isolation in pure culture of the predominating types of microorganisms.

II. A study of the ability of the organisms isolated in plan I to fix atmospheric nitrogen.

III. An investigation of the relative nitrogen-fixing ability of the soils of the plots of field A.

IV. Determination of the hydrogen-ion concentration of the soils of the plots of field A, and its possible relation to the nitrogen fixation observed in these plots.

Samples of soil for examination were collected in glass-stoppered bottles and also in 8-inch flower pots lined with a coating of paraffine. The bottled soils were taken to the laboratory for immediate examination. The moisture content of the soil in the pots was determined and they were stored in a greenhouse for use during the winter months. Every second day the pots were weighed and the loss from evaporation was made up by the addition of sterile distilled water. The bottled soil will be referred to, in this report, as series B; that in the pots will be designated as series P.

I. Bacterial flora of plot soils in field A

The first experiment undertaken was a quantitative study of the flora of the different plots of field A. Composite soil samples were taken from plots receiving nitrogen and growing legumes, from plots receiving nitrogen but not

growing legumes, from plots not receiving nitrogen but growing legumes, and from plots not receiving nitrogen and not growing legumes.

The media used for plating were Ashby's agar, meat-extract peptone agar, dextrose peptone agar, and soil-extract agar.

Ten grams of soil were added to 90 cc. of sterile physiological salt solution and shaken in a machine. Preliminary investigation suggested dilutions as follows: 1-100,000, 1-250,000 and 1-500,000. Quadruplicate plates were incubated at 25°C., and for six days where possible. Because of spreaders which obscured colony formation, meat-extract and peptone agar plates were incubated for only three days. The results, shown in table 2, indicate that the variations are inconsistent and, for any given medium, they are within the range of experimental error. They probably may be accounted for by biological variation, and are without significance.

TABLE 2
Numbers of bacteria in various soil combinations on different culture media
Millions of bacteria to a gram of dry soil

MEDIUM	BACTERIA IN SOIL COMBINATIONS*			
	N L	N N-L	N-N L	N-N N-L
Ashby's agar, Series B.....	4.06	5.44	5.25	4.06
Ashby's agar, Series P.....	8.75	6.00	9.00	11.50
Meat-extract peptone agar, Series B.....	8.57	7.07	8.90	10.42
Meat-extract peptone agar, Series P.....	4.23	7.88	3.10	1.93
Dextrose peptone agar, Series P.....	2.88	2.75	3.25	3.41
Soil-extract agar, Series P.....	3.18	1.94	4.00	3.50

* N L indicates nitrogen treatment and legume crop. N N-L indicates nitrogen treatment but non-legume crop. N-N L indicates no nitrogen but a legume crop. N-N N-L indicates no nitrogen and non-legume crop.

II. Nitrogen-fixing ability of the organisms isolated from I

A number of organisms, including several actinomyces, were isolated in pure culture from the plates in experiment I. The nitrogen-fixing ability of these organisms was tested as follows:

A heavy inoculum was placed in flasks containing a layer of sterile Ashby's agar and incubated at 25° to 28°C. for three or four days until growth was well established. Fifty cubic centimeters of sterile Ashby's solution were added aseptically and the cultures incubated at 25° to 28°C. for 14 days. This method was advocated by Ashby (1). At the end of the incubation period, total nitrogen determinations were made by the Kjeldahl method. One organism, designated as 9A, was found to fix nitrogen vigorously, the quantity averaging 10 to 12 mgm. to 100 cc. of solution, and in some cases as much as 16 mgm. to 100 cc.

The morphology of the organism, 9A, is typical for *Azotobacter*. In Ashby's solution or on Ashby's agar, cultures up to 1 week old show a predominance of

diplococcus forms, somewhat granular when stained with erythrosin or rose bengal. The cells are slightly smaller than those of three stock strains of *Azotobacter chroococcum* carried in the laboratory.

When cultivated in Ashby's solution, 9A forms a slightly wrinkled scum which varies in color from yellow to a light brown. On Ashby's agar slants, the organism produces a profuse, slimy growth. At first there is no pigment, but at the end of a week the growth becomes yellow, and darkens to a light brown in old cultures. The pigment never becomes as dark as that of the three stock cultures mentioned.

Colonies on Ashby's agar plates are large, frequently measuring 10 mm. in diameter. They are raised and slimy with a dense center. The coloration is like that of the slant cultures on Ashby's agar.

The writers feel that the organism undoubtedly belongs to the genus *Azotobacter*.

None of the other organisms fixed nitrogen in measurable quantity, although some were able to grow on nitrogen-free Ashby's medium.

III. Nitrogen-fixing ability of soil in plots of field A

The next experiment undertaken was the determination of the nitrogen-fixing ability of the soil in the different plots of field A, and the prevalence of organisms capable of fixing marked quantities of nitrogen.

Soil samples were taken from the storage pots and well mixed but not dried, the moisture content being maintained as near as possible to that of the soil when taken from the field. One-gram portions were put into 50 cc. quantities of Ashby's solution and incubated at 28°C. Controls were set up in the same manner and immediately sterilized at 15 pounds for 20 minutes, after which they were handled in exactly the same way as the cultures.

At first, incubation for three weeks was attempted but it was found that during the third week putrefaction was so rapid that the cultures were spoiled for nitrogen-fixation tests. Two weeks' incubation was employed satisfactorily for the remainder of the tests. At the end of one week some of the scum growing on the surface of the cultures was plated in Ashby's agar to determine the possible presence of nitrogen-fixing bacteria, especially of the *Azotobacter* group.

After two weeks' incubation, total nitrogen determinations were made on the cultures and controls by the Kjeldahl method.

Table 3 shows the results of these tests. One series of 1926 and one of 1928 are included as representative of the determinations. During the series of 1928, pure cultures of 9A were isolated from the plots thus indicated and were tested for nitrogen-fixing power, using Ashby's solution and incubating for three weeks at 28°C; Total nitrogen was determined by the Kjeldahl method as before. In all cases, it was found that the organism was capable of fixing substantial amounts of nitrogen. No other aerobic organism was found which could fix measurable amounts of nitrogen, although some were isolated which

could live on the nitrogen-free Ashby's medium. No isolations of anaerobic organisms were attempted.

TABLE 3
Nitrogen-fixation by soils of plots in field A
Milligrams of nitrogen to a gram of soil
North

	DATE	NITROGEN FIXED				
		Legumes West	Non-legumes West	Legumes East	Non-legumes East	
10	November, 1926	4.9+	2.4	4.7+	4.4+	Dry ground fish
		5.3	1.8	5.1	4.4	
	November, 1928	4.3+	2.3	3.0+	3.5+	
		2.7	1.3	3.8	4.0	
9	November, 1926	4.7+	1.8+	4.7+	5.4+	No nitrogen
		4.8	4.9	4.9	6.8	
	November, 1928	3.7+	2.3	4.6+	4.6+	
		3.0	1.3	3.9	3.7	
8	November, 1926	1.9	2.3	4.2+	3.6+	Ammonium sulfate
		2.1	2.6	5.4	4.7	
	November, 1928	1.5	2.1	2.2	4.5+	
		2.0	1.3	0.0	2.4	
7	November, 1926	2.2	2.1	4.8+	6.3+	No nitrogen
		2.0	2.9	4.6	4.8	
	November, 1928	3.3+	4.2+	4.5+	4.1+	
		4.0	1.9	3.4	3.7	
6	November, 1926	2.0	2.5	3.1	5.8	No nitrogen
		2.3	1.7	2.1	3.2	
	November, 1928	2.2	1.4	3.6	3.1	
		1.9	1.7	1.4	4.1	
5	November, 1926	2.2	2.1	4.2	2.2	Sodium nitrate
		2.3	1.9	1.8	2.9	
	November, 1928	2.0	4.4	2.1	4.4+	
		1.3	1.0	1.8	3.9	

The plus mark indicates the presence of organism 9A.

Table 3 shows the following results:

In general, the soil of the east half of the field has a greater nitrogen-fixing ability than that of the west half. Two apparent influences might account for this condition: the slope is

from west to east which might mean a more favorable moisture condition for *Azobacter* in the east half; and more lime has been added to the east half over a period of 20 years. The amounts of lime added to the plots are shown in table 4. A study of the pH of the different plots was made in order to investigate the possible relation of the liming to the soil acidity. This study is reported later in the manuscript.

In general, the plots showing the highest nitrogen fixation also yielded cultures of 9A. This correlation was expected. The outstanding exception was in plot 6 NLE which had a high nitrogen fixation but did not show any 9A.

The poorest nitrogen fixation was observed in strips 5, 6, and 8 which had received inorganic nitrogen compounds as fertilizer. Ammonium sulfate might be expected to lower

TABLE 4
Total lime applied to field A since 1898
Pounds an acre
North

	LIME ADDED			
	Legumes West	Non-legumes West	Legumes East	Non-legumes East
10	10,000	10,000	18,000 17,000	18,000 17,000
9	10,000	10,000	18,000 17,000	18,000 17,000
8	10,000	10,000	18,000	18,000
7	8,500 7,000	8,500 7,000	17,000 15,500	17,000 15,500
6	10,000	10,000	17,000	17,000
5	10,000	10,000	18,000 17,000	18,000 17,000

Note: The presence of two quantities in some of the squares indicates that the north and south halves of these squares have had different amounts of lime applied at certain periods.

the pH of the soil, but table 5 shows that the liming of the strips apparently had overcome any such influence. Another possible explanation is that suggested by Winogradsky(15): that other microorganisms present in the soil are stimulated by the presence of readily available organic nitrogen, and, because they grow more rapidly than the *Azotobacter*, they are able to exhaust the available supply of food, especially energy material. Strip 10 has been treated with complex organic fertilizer (dried blood and later dry ground fish) which probably is made available more slowly for microörganic consumption. Under these conditions, the *Azotobacter* seems to be able to fulfil its nitrogen-fixing function more successfully.

The presence of legume crops on the plots apparently has not influenced nitrogen fixation or the growth of 9A in the soil, since some of the legume plots showed as much fixation as any of the plots in the field.

IV. The pH of the soils of plots in field A

The soil reaction was tested by the potentiometer method, calomel and gold-leaf electrodes being used. Methods for determining hydrogen-ion concentration of soils, later compiled by Snyder (13), were used as a basis for the tests. Table 5 shows the pH determinations in 1926-27 and 1928-29. It will be observed that the figures for 1926-27 are slightly higher than those for 1928-29. This is probably because the field was last limed in the spring of 1926 and the effects had disappeared slightly in the two years elapsing between the tests.

TABLE 5
The pH of soil in plots of field A

The upper figures in each square indicate the determination made in 1926, and the lower figures, those in 1928.

North

	LEGUMES WEST	NON-LEGUMES WEST	LEGUMES EAST	NON-LEGUMES EAST
	pH	pH	pH	pH
10	6.5 5.8	6.7 5.5	6.7 6.1	6.7 6.2
9	6.6 5.8	6.5 5.7	6.6 5.7	6.6 5.8
8	... 5.8	6.2 5.8	6.5 5.7	6.4 5.7
7	6.5 6.0	6.4 6.2	6.5 6.2	6.5 6.0
6	6.2 5.8	6.2 5.8	6.4 6.0	6.3 6.0
5	... 5.9	6.1 5.7	6.1 6.0	6.1 5.9

The lowest pH, 5.5, was obtained in plot 10 NLW, which gave poor nitrogen-fixation figures and did not yield any 9A. However, the same results were observed in 1926 when the pH was 6.5 in this plot. On the whole, it seems that the pH does not explain the results obtained either in regard to nitrogen fixation or to the presence of 9A. In practically all cases, the pH was within the limit of tolerance for the growth and activity of 9A as determined by experiment in this study. The determinations were made from soil that had been stored in pots. It has been stated (13) that storage has a tendency to increase the acidity of soil; therefore the field conditions of the plots are probably more favorable than those of pots for *Azotobacter* growth and activity.

SUMMARY

Results suggest that there is sufficient nitrogen fixation in the soil of field A to account for the nitrogen reserve.

The nitrogen fixation observed in the plots is correlated with the presence of an *Azotobacter* strain designated as 9A.

Nitrogen fixation and the distribution of organism 9A appear to have remained reasonably constant over a 3-year period.

The growth of different crops, including legumes and non-legumes, has not influenced nitrogen fixation or the presence of organism 9A in the field.

Plate counts of the organisms in the soil revealed no significant information.

The soil reaction in the plots of field A does not appear to be a controlling factor in the nitrogen-fixing activity and the distribution of organism 9A; this organism tolerates a lower pH than that commonly accepted as a limiting factor for *Azotobacter* growth.

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ACTINOMYCETES IN DANISH SOILS

H. L. JENSEN¹

State Laboratory of Plant Culture, Lyngby, Denmark

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The actinomycetes have long been recognized as a constituent of the normal soil microflora. Beijerinck (1) was one of the first to call attention to the fact that the soil harbors a large number of actinomycetes, to the activities of which he ascribed the formation of humus. Soon afterward, Hiltner and Störmer (6) pointed out that these organisms often comprise 20 to 30 per cent of the total number of colonies appearing on gelatin plates in the making of bacterial counts from normal soils.

The first serious attempt to classify the actinomycetes (apart from the pathogenic forms) was not done until 1914, by Krainsky (8), but since then several contributions to an analysis of the actinomyces flora of the soil have been made by Waksman and Curtis (11, 16) and by Conn (2, 3, 4), besides many works on the general morphology and biology of the actinomycetes [for a review of the literature see Lieske (9), Waksman (11), and Ørskov (10)]. About 40 new species have been described by the aforementioned writers, and a good deal of information concerning the rôle of these organisms in biological soil processes has been gained. A great difficulty has been represented, however, by their extreme variability, which is strongly emphasized by all modern authors, especially Lieske (9), who in his very complete treatise on the morphology and biology of the actinomycetes expresses an entire disbelief in the possibility of classifying these organisms as "species." A somewhat similar, although less extreme, view is expressed by Waksman (11), who points out the possibility of classifying them as "species-groups," that is, groups composed of a number of strains showing agreement in their most characteristic morphological and (especially) biochemical features. The present paper represents an attempt to obtain some information about the actinomyces flora of Danish soils, with special reference to the possibility of distinguishing between species-groups, in the sense of Waksman.²

¹ Now bacteriologist to the Linnean Society of New South Wales, Sydney.

² The work was originally planned as a more comprehensive study, but had to be left because of the writer's departure for Australia; this paper, together with one previously published (7), represents a summary of the preliminary results. The author's thanks are due to Dr. Selman A. Waksman, New Jersey Agricultural Experiment Station, for supplying a number of authentic cultures for comparison with those isolated from the Danish soils.

COUNTINGS OF ACTINOMYCETES IN VARIOUS SOILS

Counts of bacteria and actinomycetes were carried out on the following agar medium: dextrose, 2 gm.; casein dissolved in 10 cc. 0.1 *N* NaOH, 0.2 gm.; K_2HPO_4 , 0.5 gm.; $MgSO_4$, 0.2 gm.; $FeCl_3$, trace; agar, 15 gm.; distilled water, 1,000 cc.; pH 6.5 to 6.6. Dilutions of 1:10,000–100,000 were used according to the character of the soil. Six parallel plates were usually poured, and incubated at 25°C. for 7 days. This medium was found eminently suitable for counting and isolating the actinomycetes, and because of the thin growth, which most actinomycetes produced on it, it is also very convenient when a direct microscopical examination of the living colonies is desired; for the cultural distinction of the various strains it was found less suitable. The results of the countings are found in table 1.

It will be seen that the absolute numbers of actinomycetes, which vary from none to about 13 millions per gram, are not materially different in the different kinds of soils of similar reaction; but the reaction has a marked influence on the numbers. All the strongly acid soils, of pH 4.6 and less, have, with one single exception (forest soil 31), only very small numbers of actinomycetes. At a pH value of about 5 the numbers begin to rise, and the maximal figures occur in the region of pH 6.8 to 8.0. This is in perfect agreement with the results of Gillespie (5), Waksman and Joffe (17), and Waksman (14), who found that the critical degree of acidity for the majority of actinomycetes is pH 4.8 to 5.0, and that their optimal reaction is pH 7 to 8. Waksman (13), moreover, found the percentage of actinomycetes decreasing in strongly acid soils. This is here true only in special cases; all the typical acid peat soils—forest soils 28 and 30, moor soils 38 to 41, heath soils 49 and 50—have remarkably low percentages of actinomycetes, but otherwise there is no correlation whatever between the pH values and the percentages of actinomycetes in the total flora on the plates. Even such extremely acid soils as 1, 2, and 27 have the same percentages as the neutral and alkaline soils. Furthermore, the percentages are mostly of the same order of magnitude as those found by Hiltner and Störmer (6), Waksman and Curtis (16), and Conn (2), although some excessive figures occur—soils 14, 19, 23, and 54—which were only found by Waksman and Curtis in certain subsoils.

The soil reaction thus to a marked extent controls the development of actinomycetes in the soil. This statement seems to be no more applicable to actinomycetes than to the bacteria (or at least to those bacteria which develop on the agar medium), since the relative numbers of actinomycetes were affected only in special cases, viz., in the strongly acid peat soils. It must be left to further investigations to decide what is the cause of the relative absence of actinomycetes in this soil type. It is possible that the aeration, which is usually poor in these soils, might play a rôle; however, the results recorded in table 2, where an acid peat soil was kept in a moist, loose, and well-aerated condition, do favor this hypothesis.

As a further test of the influence of reaction on numbers of actinomycetes, the following experiment was carried out: Three more or less acid soils received additions of CaCO_3 (the strongly acid peat soil, 4 per cent; the two

TABLE 1
Numbers of actinomycetes in various soils

ANALYSIS NUM- BER	SOIL	ACTINOMYCETES, MILLIONS PER GM. OF SOIL	ACTINOMYCETES, PER CENT OF TOTAL	pH OF SOIL	ANALYSIS NUM- BER	SOIL	ACTINOMYCETES, MILLIONS PER GM. OF SOIL	ACTINOMYCETES, PER CENT OF TOTAL	pH OF SOIL
<i>Field soils</i>					<i>Forest soils—Concluded</i>				
1	Humus, permanent grass	0.12	29.3	3.76	34	Sand	1.68	38.2	5.65
2	Clay	0.02	20.1	3.97	35	Mold	0.54	8.4	6.06
3	Clay	0.24	13.9	4.96	36	Mold	0.51	8.6	6.32
4	Sand	1.82	40.8	5.09	37	Loam	6.37	23.8	7.75
5	Sand	2.72	23.7	5.56	<i>Moor soils</i>				
6	Loam	2.37	32.0	5.92	38	Sphagnum peat	0.002	0.2	3.62
7	Humus	8.11	42.1	6.09	39	Mud	0.003	0.3	4.17
8	Loam	2.80	25.9	6.22	40	Sphagnum peat	0.01	2.5	4.24
9	Loam	3.51	18.3	6.70	41	Low-moor peat	0.07	4.6	4.26
10	Sand	2.22	14.9	6.78	42	Sphagnum peat, cultivated	0.21	19.9	4.58
11	Loam	5.83	24.3	6.85	43	Sphagnum peat, cultivated	0.18	4.3	5.14
12	Loam	3.79	22.7	6.86	44	Low-moor peat	1.19	33.5	5.20
13	Sand	3.53	15.7	6.91	45	Low-moor peat, cultivated	0.81	24.0	5.64
14	Sand	8.32	66.0	6.93	46	Meadow	6.76	39.1	6.90
15	Sand	2.87	16.3	6.98	47	Fen	4.00	15.4	7.08
16	Sand	2.56	16.3	7.03	48	Low-moor peat	9.67	24.6	7.61
17	Loam	3.68	20.9	7.15	<i>Heath soils</i>				
18	Sand	2.51	15.9	7.16	49	Dry peat	0	0	3.85
19	Humus	4.03	51.7	7.20	50	Dry peat	0.01	3.0	4.10
20	Loam	1.12	10.3	7.23	<i>Marsh soils</i>				
21	Loam	3.47	16.9	7.26	51	Clay	2.81	28.2	5.12
22	Loam	2.76	17.1	7.32	52	Sandy loam	2.16	25.0	6.12
23	Sand	12.82	73.3	7.40	53	Clay	1.14	24.2	6.82
24	Loam	4.12	26.0	7.50	<i>Various uncultivated soils</i>				
25	Loam	3.90	24.7	7.52	54	Sand	12.76	58.2	7.55
26	Loam	3.24	29.2	8.35	55	Sand	12.76	24.4	7.95
<i>Forest soils</i>					56	Loam	3.92	6.8	8.08
27	Mold	0.04	23.8	3.34					
28	Peat	0.01	1.9	3.86					
29	Mold	0.07	9.8	4.12					
30	Peat	0	0	4.32					
31	Mold	1.70	43.7	4.45					
32	Sand	1.07	23.5	4.97					
33	Clay	1.76	24.8	5.54					

mineral soils, each 1 per cent), and soil portions with and without lime were kept at 25°C. for about 3 months, during which the moisture content was kept as close to the optimum as possible, and the numbers of actinomycetes were determined several times. The results are given in table 2. They agree perfectly with the expectation: the strongly acid peat soil with the addition of lime shows an enormous increase in actinomycetes: the acid sand soil, poor in organic matter, shows a rather small, although definite increase: and finally the faintly acid loam shows a hardly significant increase. The relative numbers of actinomycetes in the two last soils are distinctly lowered by the lime, because of the very marked stimulation of the bacteria. The enormous development of actinomycetes in acid peat soil with lime speaks strongly in

TABLE 2
Influence of CaCO₃ on numbers of actinomycetes in acid soils

SOIL	PERIOD OF INCUBATION	WITHOUT CaCO ₃			WITH CaCO ₃		
		Number of actino- mycetes*	Actino- mycetes per cent†	pH	Number of actino- mycetes*	Actino- mycetes per cent†	pH
Heath soil 49	days						
	Start	No actinomycetes			(0)	—	—
	15	on casein agar.			18.3	22.0	7.52
	45	<i>Act. acidophilus</i>			84.9	21.1	7.47
	75	(7) present			122.9	29.6	7.59
Field soil 4	Start	1.8	40.8	5.09	1.8	40.8	—
	15	3.1	61.0	4.71	8.0	35.0	7.57
	90	2.7	57.6	4.42	10.2	23.4	7.50
Field soil 6	Start	2.4	32.0	5.92	2.4	32.0	—
	15	2.7	24.0	6.04	3.0	15.5	7.72
	90	2.9	36.2	5.78	3.5	20.4	7.62

* Millions per gm. of soil.

† Percentage of numbers of bacteria + actinomycetes on plates.

favor of the theory of Waksman (15), that several actinomycetes, if suitable reaction and good aeration are provided, are capable of decomposing soil "humus;" on the other hand we must admit that according to this theory one might expect to find especially large numbers of actinomycetes in neutral and alkaline humus soils. This is, however, not the case (table 1).

CULTURAL STUDIES OF ISOLATED ORGANISMS

About 90 strains of actinomycetes were isolated from the aforementioned soils, and attempts were made to separate them into species-groups and to identify them with previously described forms. The following characters were studied:

- I. Presence or absence of spirals in aerial mycelium on dextrose-casein-agar.
- II. Cultural characters on the following media:^a
 1. *Czapek's agar*. Saccharose, 30 gm.; NaNO₃, 2 gm.; K₂HPO₄, 1 gm.; MgSO₄, 0.5 gm.; KCl, 0.5 gm.; FeSO₄, trace; agar, 15 gm.; distilled water, 1,000 cc.; pH 7.
 2. *Glycerin-agar*. Glycerin 10 gm.; asparagin, 1 gm.; K₂HPO₄, 0.5 gm.; agar, 15 gm.; pH 7.
 3. *Starch-casein-agar*. Soluble starch, 10 gm.; casein dissolved in 1 N NaOH, 1 gm.; K₂HPO₄, 0.5 gm.; MgSO₄, 0.5 gm.; agar, 15 gm.; distilled water, 1,000 cc.; pH 7. This medium was found especially favorable in many cases, allowing a very abundant and yet characteristic growth with strong formation of a characteristically colored aerial mycelium of nearly all strains.
 4. *Nutrient agar* with addition of 2 per cent glycerin and 0.1 per cent K₂HPO₄. This medium gave an excellent growth and in many cases a characteristically colored vegetative mycelium and soluble pigments, but often no aerial mycelium.
 5. *Gelatin*. 15 per cent gold label gelatin in distilled water; reaction adjusted to pH 7.
 6. *Potato plugs*.
 7. *LOEFFLER'S Blood Serum*.

All cultures were made as slope cultures in ordinary test tubes and incubated at 25°C. Observations were made for a period of about 6 weeks.

Among the strains of actinomycetes tested in this way the following species-groups could be recognized:

- A. No brown pigment in protein-media.
 1. Red or blue pigments in synthetic media; marked reduction of nitrates:
Actinomyces violaceus-ruber.
 2. No red or blue pigments.
 - a. Typical golden pigment in all synthetic media:
Actinomyces fulvissimus.
 - b. Pigment not typical; abundant aerial mycelium.
 - I. Aerial mycelium on synthetic media dark slate-gray; lemon- or sulfur-yellow pigments sometimes formed.
 - α. Vegetative mycelium on Czapek's agar light-colored:
Actinomyces cellulosa.
 - β. Vegetative mycelium on Czapek's agar turning dark:
Actinomyces olivaceus.
 - II. Aerial mycelium greenish or yellowish gray; very rapid liquefaction of gelatin and blood-serum.
 - α. Aerial mycelium greenish:
Actinomyces griseus.
 - β. Aerial mycelium yellowish:
Actinomyces griseoflavus.
- B. Typical brown pigment in protein-media ("chromogenus" species).
 1. Deep brown growth and pigment in all synthetic media:
Actinomyces phaeochromogenus.

^a A much larger variety of media were tested, but finally those here mentioned were selected as giving the most satisfactory results.

2. Pigment in synthetic media of other color or absent.

a. Aerial mycelium absent or in traces; typical red vegetative mycelium:

Actinomyces bobili.

b. Aerial mycelium more or less abundant.

I. Typical red pigment in Czapek's agar:

Actinomyces erythrochromogenus.

II. Pigment not red.

 α . Aerial mycelium rose to cinnamon-brown:*Actinomyces roseus.* β . Aerial mycelium abundant, characteristic lead-grey; light brown pigment in synthetic media:*Actinomyces diastatochromogenus.*

A brief description of these groups is given in the following:

Actinomyces griseus Krainsky, emend. Waksman and Curtis. Six strains + one authentic examined. *Spiral formation*: none. Growth on *Czapek's agar*: Fair; vegetative mycelium scant, thin, first colorless, later yellowish gray; aerial mycelium abundant, thick, dusty layer of a characteristic greenish gray color, sometimes with a yellowish tinge; a faint yellowish gray soluble pigment is sometimes formed. *Glycerin-agar*: Scant growth; vegetative mycelium thin, colorless; aerial mycelium thin, first white, later yellowish green. *Starch-casein-agar*: Abundant and characteristic growth; vegetative mycelium spreading, cream-colored to greenish gray; aerial mycelium formed early and abundantly, thick dusty layer of a strong greenish gray color; olive-green soluble pigment sometimes formed. *Nutrient agar*: Abundant growth; vegetative mycelium cream-colored; aerial mycelium formed after 15 to 20 days, first white, later greenish gray to yellowish green; faint yellowish brown, soluble pigment in old cultures. *Potato*: Strong and characteristic growth; vegetative mycelium abundant, folded, first cream-colored, later yellowish or grayish green; aerial mycelium abundant, dusty, grayish green; plug colored gray. *Gelatin*: vegetative mycelium cream-colored, later greenish gray; aerial mycelium scant, white, later yellowish gray; slant of gelatin completely liquefied in 4 to 6 days; smell of ammonia; no pigment. *Loeffler's blood serum*: uncharacteristic growth; no aerial mycelium or pigment; serum almost completely liquefied in 15 days. Most cultures possess a very strong "soil" odor, some strains a remarkably pungent smell of musty straw. *Occurrence*: common; although not in large numbers.

Actinomyces griseoflavus Krainsky. Two strains examined. *Spiral formation*: none. *Czapek's agar*: good growth; vegetative mycelium flat, spreading, first colorless, later yellowish gray; aerial mycelium abundant, thick, dusty, first white, later characteristic yellowish gray, often forming a mosaic of white and yellowish gray patches; no pigment. *Glycerin-agar*: fair growth; vegetative mycelium flat, first colorless, later pale yellow to yellowish gray; aerial mycelium smooth, first white, later grayish yellow, with net or ring formation; no pigment. *Starch-casein-agar*: very good growth; vegetative mycelium flat, spreading, first colorless, later cream-colored; aerial mycelium abundant, thick, white to grayish yellow with distinct net formation; no pigment. *Nutrient agar*: abundant, but uncharacteristic growth; vegetative mycelium raised, folded, cream-colored to light yellowish brown; aerial mycelium in patches, white to yellowish gray; pigment absent or faint yellowish brown. *Potato*: enormously strong growth, plug after 8 to 10 days completely covered by tufts of mycelium 4 to 6 mm. high; vegetative mycelium raised, folded, cream-colored; aerial mycelium abundant, dusty, white to yellowish gray, sometimes with a pink tinge. *Gelatin*: vegetative mycelium smooth, white to cream-colored; aerial mycelium well developed, white to light yellowish gray; gelatin completely liquefied in 6 to 7 days; no pigment. *Loeffler's blood serum*: uncharacteristic growth; serum largely liquefied in 15 days. *Occurrence*: not common. This group resembles the preceding one very much, but can be distinguished from it by the characteristic grayish yellow of its aerial mycelium which never assumes the distinct green shade of the *griseus* group.

Actinomyces cellulosa Krainsky. Five strains examined. *Spiral formation*: none. *Czapek's agar*: fair and characteristic growth; vegetative mycelium thin, spreading deep into medium, of a characteristic transparent appearance, first colorless, in old cultures sometimes lemon-yellow; aerial mycelium abundant, thick, crusty, often with zone-formation, first light gray, later deep slate gray; pigment mostly absent, sometimes bright lemon-yellow. *Glycerin-agar*: fair, but less characteristic growth; vegetative mycelium narrow, first white, later light gray to yellowish gray or almost black; aerial mycelium abundant, first white, later dark slate-gray or grayish brown; pigment absent or faint lemon-yellow to yellowish brown. *Starch-casein-agar*: good growth; vegetative mycelium smooth, first colorless or cream-colored, later yellowish brown; aerial mycelium variable, often abundant, slate gray; pigment absent or light yellowish brown. *Nutrient agar*: abundant and characteristic growth; vegetative mycelium spreading, much folded, first cream-colored, later sulfur-yellow; aerial mycelium formed late, but becomes abundant, first thin and white, later of the characteristic slate-gray color; no pigment. *Potato*: abundant and characteristic growth; vegetative mycelium raised, folded, cream-colored, later often sulfur-yellow; aerial mycelium abundant, first white, later dark slate-gray, often with yellow edges. *Gelatin*: vegetative mycelium thin, yellowish gray to grayish black; aerial mycelium in patches, grayish white to gray-brown; gelatin completely liquefied in 5 to 8 days; no pigment. *Loeffler's blood serum*: vegetative mycelium cream-colored; aerial mycelium white to light gray; no pigment; serum completely liquefied in 20 days. *Occurrence*: very common.

Actinomyces olivaceus Waksman. Seven strains + one authentic examined. *Spiral formation*: mostly none, sometimes a few imperfect, open spirals. *Czapek's agar*: fair growth; vegetative mycelium thin, spreading into medium, first colorless, later yellowish gray, dark gray, or almost black; aerial mycelium abundant, smooth, dusty, often forming a mosaic of white and yellowish gray patches; pigment absent or light lemon-yellow. *Glycerin agar*: fair growth; vegetative mycelium narrow, growing into medium, first light gray, later black, often with yellow tinge, aerial mycelium abundant, smooth, first white, later dark gray; pigment absent or light yellow. *Starch-casein-agar*: good growth; vegetative mycelium flat, smooth, dark gray; aerial mycelium abundant, smooth, slate-gray, often with mosaic-formation; pigment absent or faint yellow. *Nutrient agar*: good growth; vegetative mycelium raised, spreading, cream-colored; aerial mycelium thin, in patches, white to yellowish gray; faint yellowish pigment. *Potato*: abundant growth; vegetative mycelium raised, folded, cream-colored; aerial mycelium abundant, dusty, grayish white to slate-gray or dark yellowish gray with yellow edges (much like *Act. cellulosa*). *Gelatin*: vegetative mycelium thin, cream-colored to yellowish gray; aerial mycelium thin, gray and white patches; gelatin completely liquefied in 6 to 8 days; no pigment. *Occurrence*: very common. Closely related to the previous group, from which those strains which do not produce an intensely colored growth on Czapek's agar are difficult to distinguish; most characteristic is perhaps the mosaic formation in the aerial mycelium.

Actinomyces violaceus-ruber Waksman and Curtis. Three strains + one authentic examined. *Spiral formation*: numerous spirals. *Czapek's agar*: good growth; vegetative mycelium thin, spreading, first colorless, then red-violet, in old cultures deep blue-violet; aerial mycelium abundant, smooth, light gray; pigment first light pink-violet, later sky-blue. *Glycerin-agar*: good growth; vegetative mycelium thin, first white, with red and violet spots, later deep violet-blue. *Starch-casein-agar*: abundant growth; vegetative mycelium flat, spreading, first pink, later turning deep blue; aerial mycelium in patches, white to grayish blue; pigment dark blue. *Nutrient agar*: abundant growth; vegetative mycelium raised, folded, first cream-colored, later turning deep blue-green; aerial mycelium thin, smooth, white to ash-gray; olive-green pigment in old cultures. *Potato*: good growth; vegetative mycelium spreading, folded, blue; aerial mycelium in patches, gray, turning dark violet; dark blue pigment. *Gelatin*: vegetative mycelium cream-colored with red spots; aerial mycelium scant, white patches; slow liquefaction (complete after 25 days); faint red-brown pigment in old cultures. *Loeffler's blood serum*: vegetative mycelium flat, spreading, violet-blue;

no aerial mycelium; no pigment; trace of liquefaction after 25 days. *Occurrence*: fairly common.

Actinomyces fulvissimus n. sp. Six strains examined. *Microscopical appearance*: vegetative mycelium without any special characteristics; aerial mycelium of short, straight, often trifurcated hyphae, 1.0 to 1.2 μ broad; no spiral formation; branches of hyphae break up into oidia, 1.0 to 1.2 \times 1.2 to 1.5 μ . *Czapek's agar*: good growth (one strain very scant), vegetative mycelium flat, narrow, first light golden, later deep orange to red-brown; aerial mycelium scant, sometimes almost absent, first white, later light grayish brown; pigment very characteristic, bright golden to orange. *Glycerin-agar*: good growth; vegetative mycelium narrow, raised, smooth, golden to dark bronze; aerial mycelium scant, in patches, white to light cinnamon-brown; pigment intensely golden to orange. *Starch-casein-agar*: good growth; vegetative mycelium spreading, folded, yellowish brown; aerial mycelium abundant, smooth, lead-gray; pigment dull yellow to orange. *Nutrient agar*: good growth; vegetative mycelium raised, finely wrinkled, deep red-brown; no aerial mycelium; brownish yellow pigment. *Potato*: good growth; vegetative mycelium raised, much wrinkled, rust-brown; aerial mycelium absent or traces of white; pigment gray to faint lemon-yellow. *Gelatin*: vegetative mycelium narrow, smooth, yellowish brown to red-brown; no aerial mycelium; no pigment; gelatin completely liquefied in 10 to 12 days. *Loeffler's blood serum*: vegetative mycelium red-brown; no aerial mycelium; yellowish pigment; no liquefaction. The characteristic golden pigment is formed in nearly all media in which the organism grows, but becomes most typical and attains its greatest brightness in synthetic agar media; it has indicator properties, turning red in strongly acid solutions. *Occurrence*: extremely common in Danish soils; easily recognized on agar plates by its bronze-colored colonies, surrounded by haloes of bright yellow pigment.

Actinomyces roseus Krainsky, emend. Waksman and Curtis. Six strains + one authentic examined. *Spiral formation*: present, sometimes abundant. *Czapek's agar*: scant to strong growth; vegetative mycelium thin, white, sometimes (e.g. authentic culture from Waksman) with age becoming greenish black with metallic lustre; aerial mycelium thin, sometimes in patches, light rose; no pigment. *Glycerin-agar*: fair growth; vegetative mycelium flat, spreading into medium, white to pale pink, in one strain grayish green; aerial mycelium smooth, rose to cinnamon-brown; no pigment. *Starch-casein-agar*: good growth; vegetative mycelium spreading into medium, yellowish brown to greenish gray; aerial mycelium abundant, thick, smooth layer of a characteristic rose color; no pigment. *Nutrient agar*: abundant growth; vegetative mycelium superficially spreading, cream-colored to red-brown, in two strains of a characteristic green color; aerial mycelium scant, white, formed late; brown pigment. *Potato*: good to abundant growth; vegetative mycelium spreading, flat or folded, yellowish gray to greenish black in two strains; aerial mycelium scant to abundant, first white, later rose-colored; brownish gray pigment. *Gelatin*: vegetative mycelium narrow, folded, yellowish brown to gray-green; aerial mycelium scant, white to light rose; brown pigment; slow liquefaction. *Occurrence*: fairly common, though not in large numbers.

Actinomyces bobili Waksman and Curtis. Six strains + one authentic examined. *Spiral formation*: none. *Czapek's agar*: good growth; vegetative mycelium raised, spreading, somewhat folded, pale rose to coral-red; with colorless edges; no aerial mycelium; no pigment. *Glycerin agar*: good growth, in appearance like the previous, blood-red; no aerial mycelium or only traces of white; mostly no pigment, sometimes pale rose. *Starch-casein-agar*: good, but less characteristic growth; vegetative mycelium superficially spreading, grayish rose, sometimes with small tufts of grayish white aerial mycelium; grayish orange pigment. *Nutrient agar*: abundant and characteristic growth; vegetative mycelium superficially spreading, coarsely folded (lichenoid), characteristically coral-red; no aerial mycelium; deep brown pigment; when glycerin is omitted from the medium, a comparatively scant and quite uncharacteristic yellowish brown growth is obtained. *Potato*: fair growth; vegetative mycelium raised, wrinkled, grayish brown to coral-red; aerial mycelium absent or traces of white; pigment grayish to black. *Gelatin*: vegetative mycelium orange, sometimes with patches of

white aerial mycelium; brown pigment; fairly rapid liquefaction, almost complete after 15 days. *Occurrence*: common soil organism: easily recognized on plates of casein-agar by its colonies which resemble drops of sealing wax.

Actinomyces diastatochromogenus Krainsky. Six strains examined. *Spiral formation*: abundant. *Czapek's agar*: good growth; vegetative mycelium superficially spreading, first colorless, later cream-colored to yellowish brown; aerial mycelium abundant, formed late, first white, later ash-gray; yellowish to light brown pigment. *Glycerin agar*: fair growth; vegetative mycelium spreading into medium, colorless to cream-colored; aerial mycelium less abundant than on previous medium, white to light gray; no pigment. *Starch-casein-agar*: abundant and characteristic growth; vegetative mycelium superficially spreading, yellowish gray; aerial mycelium abundant, smooth, first white, later deep lead-gray, with characteristic net formation; grayish yellow to yellowish brown pigment. *Nutrient agar*: abundant growth; vegetative mycelium spreading, yellowish to grayish brown; aerial mycelium absent or scant, white; light coffee-brown pigment. *Potato*: fair growth; vegetative mycelium spreading, finely wrinkled, first yellowish gray, later grayish black, with or without traces of white aerial mycelium; black pigment. *Gelatin*: vegetative mycelium cream-colored to yellowish brown; aerial mycelium scant, white; brown pigment; gelatin fairly rapidly liquefied. *Loeffler's blood serum*: scant and uncharacteristic growth; dark yellowish brown pigment; no liquefaction. *Occurrence*: very common.

Actinomyces erythrochromogenus Krainsky. Two strains examined. *Spiral formation*: abundant. *Czapek's agar*: fair growth; vegetative mycelium thin, first cream-colored, later turning dark violet to purple; aerial mycelium smooth, light gray; red-violet pigment. *Glycerin-agar*: fair growth; vegetative mycelium narrow, growing into medium, first light red-violet, later turning dark red; aerial mycelium thin, light gray to red-gray; yellowish red pigment. *Starch-casein-agar*: good growth; vegetative mycelium flat, spreading, dark violet; aerial mycelium smooth, light gray; purplish gray pigment. *Nutrient agar*: good growth; vegetative mycelium raised, wrinkled, yellowish gray to light brown; aerial mycelium smooth, light gray; deep brown pigment. *Potato*: good growth; vegetative mycelium raised, wrinkled, first yellowish gray, later almost black; deep black pigment. *Gelatin*: vegetative mycelium finely wrinkled, yellowish to light purple, with thin patches of aerial mycelium; brown pigment; very slow liquefaction. *Occurrence*: not common.

Actinomyces pheochromogenus Conn. One strain examined. *Spiral formation*: present. *Czapek's agar*: good growth; vegetative mycelium growing deep into medium, first red-brown, later nearly black; aerial mycelium well developed, smooth, first pure white, later with brownish tinge; dark brown pigment. *Glycerin agar*: good growth; vegetative mycelium raised, folded, brownish black; no aerial mycelium; brown pigment. *Starch-casein-agar*: like on previous medium, but with thin white aerial mycelium. *Nutrient agar*: good growth; vegetative mycelium raised, smooth, red-brown, later turning almost black; no aerial mycelium; brown pigment. *Potato*: fair growth; vegetative mycelium raised, wrinkled, black; no aerial mycelium; black pigment. *Loeffler's blood serum*: scant growth; vegetative mycelium brownish gray; no aerial mycelium; brown pigment; no liquefaction. *Occurrence*: not common.

Besides these, some strains were found, which probably belonged to or were closely related to the following groups: *Act. aureus* Waksman and Curtis, *Act. Halstedii* Waksman and Curtis, *Act. olivochromogenus* Waksman, and *Act. viridochromogenus* Krainsky. The rest of the cultures—the majority—could not be identified, although several of them could be classified in more or less defined species-groups. The time available for the experiments did not, however, allow a thorough study of these groups, of which only one, viz., the *Act. fulvissimus* already described, was considered sufficiently well defined to be described and named as a new species-group.

PHYSIOLOGICAL STUDIES OF ISOLATED ORGANISMS

Attempts were made to distinguish, by means of the following physiological tests, between the various species-groups recognized through cultural tests, in order to see how far such physiological tests would be of value for the characterization of the non-identified groups: utilization of carbohydrates; acid formation from carbohydrates; reduction of nitrate; diastatic activity; proteolytic activity; resistance to hydrogen-ion concentration.

The utilization of carbohydrates was studied by growing the organisms for 10 days⁴ at 25°C. on the following solution: carbohydrate, 10 gm. (cellulose supplied as a strip of pure Swedish filter paper); NaNO₃, 2 gm.; K₂HPO₄, 1 gm.; MgSO₄, 0.5 gm.; distilled water, 1,000 cc. At the end of the experiment, tests were made for NO₂ formation by means of Gries' reagent, acid formation by addition of bromo-thymol-blue (in some cases also by electro-metric determination of the reaction), invertase production in saccharose solution by Fehling's reagent, and diastatic activity in starch solution by means of iodine solution.

The utilization of various carbon compounds is shown in table 3. It is seen at once that this gives hardly any differentiation among the groups. Dextrose, galactose, maltose, and starch are the best sources of carbon for nearly all groups, and the only indication of an intergroup distinction is, that the *olivaceus* group hardly utilizes maltose at all and grows poorly on dextrose, and that the *violaceus-ruber* and *diastatochromogenus* groups are the only ones which grow well on lactose and rhamnose. The value of these features, however, seems nullified by the circumstance that the intragroup variation in several cases is equally large, ranging from 0 to 5 in the *griseus* group on arabinose, 2 to 4 in the *olivaceus* group on glycerin and galactose and the *violaceus-ruber* group on dextrose. The table further shows that several strains, especially of the *violaceus-ruber* group, are able to utilize cellulose, although none of the strains here dealt with produced any very considerable growth, even after long incubation. A few non-identified strains, isolated from soil with addition of cellulose, made a good growth and caused a marked disintegration of the filter paper within a few weeks.

The results of the tests for nitrites and acids are given in table 4. The *violaceus-ruber* group shows decidedly the strongest nitrite-formation [as has also been recorded by Waksman (11)]; and in the *griseus*, *griseoflavus*, *cellulosae*, and *olivaceus* groups this property is almost lacking; and, finally, in the rest of the strains it is quite variable and does not characterize any particular group.

The tests for acid formation gave an interesting result. In most papers on actinomycetes (for example, Waksman (12, 17) it is stated that these organisms do not form organic acids from carbohydrates; this was also the case here

⁴ Except the cellulose-cultures, which were incubated for 4 months.

TABLE 3
Utilization of various carbon-compounds by actinomycetes*

SPECIES-GROUP	STRAIN	GLYCERIN	ERYTHRIT	MANNITE	ARABINOSE	XYLOSE	RHAMNOSE	DEXTROSE	LEVULOSE	GALACTOSE	SACCHAROSE	MALTOSE	LAKTOSE	RAFFINOSE	INULIN	STARCH	CELLULOSE
<i>Actinomyces griseus</i>	A. L. VIII.	2	1	4	5	2	0	3	2	3	1	3	0	0	1	4	0
	H. VI.	3	0	3	0	2	1	3	1	3	1	4	1	1	2	5	0
	M. III.	—	1	3	0	0	1	3	1	3	2	2	0	1	2	4	0
	V. III.	3	1	2	0	2	1	3	2	3	1	3	0	0	2	5	0
	W. aut.	2	—	5	2	—	1	4	—	5	5	4	1	1	2	5	—
<i>Actinomyces griseo-flavus</i>	A. XVIII.	5	1	5	2	2	1	5	4	5	2	4	2	0	1	5	0
	He J. IV.	4	1	2	2	2	1	5	4	5	2	5	2	0	2	5	1
<i>Actinomyces cellulosae</i>	Sp. I.	2	1	1	1	2	1	4	4	4	1	4	0	0	1	5	1
	A. V.	5	1	2	3	2	2	3	2	4	2	5	0	1	1	5	2
	A. X.	4	1	2	—	2	0	4	3	3	1	4	0	1	1	4	2
	A. L. III.	3	1	1	1	2	0	3	3	4	1	3	0	0	2	5	1
<i>Actinomyces olivaceus</i>	Sp. III.	3	0	1	0	1	1	—	1	3	0	1	0	0	—	4	1
	B. XV.	4	0	1	4	2	1	2	1	2	1	0	0	0	1	2	2
	L.	4	1	2	3	0	0	2	0	3	1	1	0	1	2	4	3
	L. M. II.	2	0	—	0	2	0	—	—	4	1	0	0	0	2	5	1
	W. aut.	2	0	2	1	2	0	1	—	4	1	0	0	0	—	5	—
<i>Actinomyces violaceus-ruber</i>	L. II.	3	1	3	3	2	2	4	1	3	1	4	4	2	1	5	3
	L. III.	2	—	3	3	3	3	2	1	3	1	4	3	2	2	4	3
	V. G. III.	2	1	2	2	2	3	3	0	4	2	4	2	3	2	4	1
	W. aut.	1	—	2	2	—	—	2	—	1	1	—	3	1	—	2	—
<i>Actinomyces fulvis-simus</i>	H. I.	3	1	1	3	4	0	4	3	3	2	4	0	4	2	3	0
	T. XIV.	2	1	1	3	2	3	4	2	4	3	3	0	3	3	5	0
	H. X.	3	1	1	2	2	0	4	2	1	2	—	3	2	2	4	0
<i>Actinomyces roseus</i>	B. V.	5	0	1	0	0	0	3	1	2	3	3	2	3	2	5	0
	R. IV.	5	1	0	1	0	0	4	1	3	2	4	3	1	1	3	0
	U. II.	—	0	—	—	—	0	—	—	—	4	—	—	3	—	—	1
	W. aut.	1	0	0	3	1	0	3	—	1	1	—	—	0	—	4	—
<i>Actinomyces bobili</i>	U. G.	2	1	3	1	2	2	2	2	3	3	2	2	3	3	2	2
	W. aut.	—	0	1	0	2	1	3	—	3	2	3	3	4	—	3	2
<i>Actinomyces dia-statochromogenus</i>	B. IV.	4	0	5	3	2	4	3	1	4	—	4	4	0	5	4	2
	A. XVI.	4	1	4	1	1	3	2	0	—	4	3	3	2	5	4	—
<i>Actinomyces pheochromogenus</i>	H. b.	2	0	1	0	2	1	1	0	1	1	1	1	3	1	1	0

* The amount of growth is here and in the subsequent tables indicated by the following characters: 0 = no growth. 1 = trace of growth. 2 = scant. 3 = fair. 4 = good. 5 = excellent.

TABLE 4
Formation of organic acids from carbohydrates and of NO_2 from NO_3 by actinomycetes in synthetic solution

ORGANISM	ACID FORMED FROM CARBOHYDRATES	NO_3 FORMATION FROM NaNO_3	REACTION DETERMINATION IN CASE OF POSITIVE ACID FORMATION		
			Carbohydrate	pH	pH sterile solution
<i>Actinomyces griseus</i> , 5 strains.....	None	None or trace	—	—	—
<i>Actinomyces griseoflavus</i> , 2 strains.....	None	None or trace	—	—	—
<i>Actinomyces cellulosa</i> , 4 strains.....	None	None or trace	—	—	—
<i>Actinomyces olivaceus</i> , 5 strains.....	None	None	—	—	—
<i>Actinomyces</i> :					
HI, HX, T XIV.....	None	None or trace	—	—	—
RV.....	Saccharose, Mannite	None to faint	Saccharose	4.5	7.2
<i>Act. violaceus-ruber</i> :					
L. II.....	Glycerin, Arabinose, Levulose	Very strong	Glycerin	5.9	7.2
L. III.....	Glycerin, Rhamnose, Levulose	Very strong	Glycerin	5.8	7.2
			Levulose	6.2	6.6
V. G. III.....	None	Very strong	—	—	—
<i>Act. roseus</i> :					
B. V.....	Saccharose, Galactose	None to faint	Saccharose	5.5	7.2
V. II.....	Saccharose	None to faint	Saccharose	5.5	7.2
<i>Act. bobili</i> :					
V. XII.....	None	None to faint	—	—	—
V. G.....	Saccharose	None to distinct	Saccharose	5.3	7.2
<i>Act. Halstedii</i> :					
B. IX.....	Saccharose	Mostly none, sometimes faint	Saccharose	6.1	7.2
B. XIX.....	Mannite, Saccharose		Saccharose	6.0	7.2
<i>Act. aureus</i> L. s. (?).....	None	None	—	—	—
<i>Act. erythrochromogenus</i> T. IV.....	Dextrose, Saccharose	None, sometimes faint	Saccharose	5.6	7.2
<i>Act. diastatochromogenus</i> A. XVI.....	Lactose, Dextrose, Maltose	Strong	Dextrose	6.0	7.2

with most of the cultures, but many of the strains (several of the non-identified besides those here mentioned) show an undoubted formation of acids from various carbohydrates; this acid formation varies both with the type of the organism and the nature of the carbon compound: no strain of the *griseus*, *griseoflavus*, *cellulosae*, or *olivaceus* groups showed any acid formation, and in no case was acid formation observed in solutions of erythrit, inulin, and starch. The formation of acid was constantly accompanied by a more or less vigorous nitrite formation, whereas the reverse did not hold. It is noteworthy that in no case was any turbidity of the cultures, which might suggest bacterial infection, observed.

The results of tests for diastase and invertase production are given in table 5 and show that the *griseus*, *griseoflavus*, *cellulosae* and *olivaceus* groups together with the *fulvissimus* group are the most powerful starch decomposers, although

TABLE 5
Diastase and invertase production in starch and saccharose solution

ORGANISMS	STARCH REACTION	REDUCTION OF Fehling's solution
<i>Act. griseus</i> , 5 strains	None	None
<i>Act. griseoflavus</i> , 2 strains	None	None
<i>Act. cellulosae</i> , 5 strains	None	None
<i>Act. fulvissimus</i> , 3 strains	None	Faint
<i>Act. olivaceus</i> , 5 strains	None	None
<i>Act. violaceus-ruber</i> , 3 strains	Trace or faint	None
<i>Act. diastatochromogenus</i> , 3 strains	None or trace	Strong
<i>Act. bobili</i> , 2 strains	Good	Strong
<i>Act. Halstedii</i> (?), 2 strains	None or trace	—
<i>Act. aureus</i> , 2 strains	Strong	None
<i>Act. pheochromogenus</i>	Strong	None
<i>Act. erythrochromogenus</i>	Trace	—

the *diastatochromogenus*, *Halstedii* (?), and *erythrochromogenus* groups follow them closely, and the rest of the "chromogenus" actinomycetes (*bobili*, *aureus* (?), and *pheochromogenus*) seem less active. The test with Fehling's solution shows that only two groups, *Act. bobili* and *diastatochromogenus*, produce appreciable amounts of reducing sugars in the medium; the first species was also found by Waksman (11, 12) to produce invertase.

As a test for *proteolytic activities*, organisms were grown on the following solution: gelatin, 20 gm.; K₂HPO₄, 2 gm.; MgSO₄, 0.5 gm.; NaCl, 0.5 gm.; distilled water, 1,000 cc.; pH 7. The solution was used in portions of 25 cc. in 50-cc. Erlenmeyer flasks and incubated at 25°C. Four parallel flasks were used, of which two were analyzed after 10 days and two after 30 days. Ammonia-N was determined by distillation with MgO, formol-titrating N by the method of Sørensen.

Table 6 gives the results, together with records of the liquefaction of gelatin

TABLE 6
Proteolytic activity of actinomycetes in 2 per cent gelatin solution

SPECIES-GROUP	STRAIN	10 DAYS PER 25 CC. SOLUTION		30 DAYS PER 25 CC. SOLUTION		LIQUEFACTION OF	
		NH ₄ -N	Formol- titr. N	NH ₄ -N	Formol- titr. N	Gelatin	Blood serum
		mgm.	mgm.	mgm.	mgm.		
<i>Actinomyces</i> <i>griseus</i>	A. L. VIII.	3.7	18.8	14.7	34.4	Very rapid	+
	H. VI.	6.8	14.2	17.5	32.2		
	M. III.	4.0	15.3	17.7	31.9		
	V. III.	9.0	17.7	16.1	27.0		
<i>Actinomyces</i> <i>griseoflavus</i>	Hed. IV.	6.8	17.9	22.8	38.5	Very rapid	+
	A. XVIII.	7.4	16.2	21.4	34.7		
<i>Actinomyces</i> <i>cellulosae</i>	Sp. I.	3.7	16.1	14.7	27.3	Rapid	+
	A. V.	6.6	15.5	14.6	24.9		
	A. X.	3.9	16.8	13.7	27.0		
	A. L. III.	3.4	15.1	15.3	29.8		
<i>Actinomyces</i> <i>olivaceus</i>	B. XV.	1.6	11.3	6.0	28.4	Rapid	+
	L. II.	3.0	12.1	12.3	19.1		
	L. M. II.	2.5	15.1	5.6	28.9		
	W. aut.	2.7	14.2	5.8	25.3		
<i>Actinomyces</i> <i>fulvissimus</i>	H. I.	4.1	15.8	9.7	34.8	Fairly rapid	÷
	T. XIV.	0.9	7.8	4.2	24.2		
	H. X.	2.3	13.5	7.7	33.3		
<i>Actinomyces</i> <i>violaceus-ruber</i>	H. L. II	2.6	11.8	10.0	31.4	Rapid	÷
	H. L. IV.	2.8	17.2	9.0	46.9		
	V. G. III.	2.3	14.7	8.4	46.2		
<i>Actinomyces</i> <i>roseus</i>	B. V.	2.3	11.4	11.6	21.4	Slow	—
	R. IV.	2.0	11.4	8.8	18.9		
<i>Actinomyces</i> <i>bobili</i>	V. G.	1.4	9.0	3.3	19.1	Slow	÷
	W. aut.	3.2	13.3	9.0	34.7		
<i>Actinomyces</i> <i>viridochromogenus</i>	S. VI (?).	2.3	9.7	6.7	15.8	Slow	÷
	W. aut.	2.8	12.5	14.4	23.8		
<i>Actinomyces</i> <i>erythrochromogenus</i>	T. IV.	3.5	13.0	7.7	20.5	Slow	
<i>Actinomyces</i> <i>pheochromogenus</i>	H. b.	2.0	9.3	4.4	22.3	Slow	÷
<i>Actinomyces</i> <i>diastatochromogenus</i>	B. IV.	3.1	11.9	11.2	23.5	Slow	+
	A. XVI.	3.0	10.4	10.9	19.4		
<i>Actinomyces</i> <i>aureus</i> (?)	L. S.	2.1	8.8	5.8	22.6	Slow	+
Sterile solution	—	—	—	0.0	6.8	—	—

and blood serum. Although there is considerable variation among the strains within each single group, *Act. griseus*, *griseoflavus*, and *cellulosae* are seen to form a separate group of strains which split large amounts of protein, and a remarkably large proportion of this is present as ammonia-N; especially is this true of *Act. griseus*, which Waksman (11, 12) also found to be a very strongly proteolytic organism, and the closely related *Act. griseoflavus*. The next three groups of non-chromogenus actinomycetes, viz., *Act. olivaceus*, *violaceus-ruber*, and *fulvissimus* split about as much protein—two strains of *violaceus-ruber* most of all—but a distinctly smaller part of this is present as ammonia. Finally, the whole group of chromogenus actinomycetes (with one exception, the authentic strain of *Act. bobili*) shows a weaker proteolytic power and in most cases produces only small amounts of ammonia. The table further shows a fair parallelism between proteolytic power and rapidity of gelatin liquefaction, and, in agreement with the observations of Waksman (12), it shows that only the highly proteolytic strains are capable of liquefying blood serum.

RESISTANCE OF ACTINOMYCETES TO ACIDITY

All authors seem to agree that the actinomycetes as a whole are very sensitive toward acid reaction. Gillespie (5) showed that the hydrogen-ion concentration which checks the growth of *Act. scabies* in nutrient solution, is pH 4.8 to 5.2, and in accordance herewith Waksman and Joffe (17) found that *Act. violaceus ruber*, *scabies*, *griseus*, and *reticulus ruber* would produce a scant growth in solutions of pH 5, but not at pH 4.8; they further observed that when grown in a nutrient solution with dextrose as a source of carbon and ammonium sulfate as a source of nitrogen, nine species of actinomycetes would change the reaction from pH 5.8 to pH 4.4 to 4.6, one species (*Act. viridochromogenus*) even to pH 4.2. Waksman (14) further found that the limiting reaction of pH 4.8 to 5 also holds for *Act. scabies* when grown in sterile soil; some non-parasitic forms (*griseus*, *violaceus ruber*) seemed able to resist a slightly higher acidity.

An attempt was made here to differentiate between the groups of actinomycetes by determining the maximum hydrogen-ion concentration which they would tolerate, expressed as the degree of acidity arising in a physiologically acid medium. The following solution was used: dextrose, 20 gm.; NH_4Cl , 2 gm.; K_2HPO_4 , 0.2 gm.; MgSO_4 , 0.5 gm.; NaCl , 0.5 gm.; distilled water, 1,000 cc.; pH 6.5. Ten cubic centimeter portions in test tubes were used, and duplicate cultures were incubated for 15 days at 25°C., after which time the reaction was measured by means of the quinhydrone electrode.

The results, given in table 7, show some interesting features. The strains within each group seem to show far less variation in this than in any other respect here studied (the only serious discrepancy is shown by strain LII of the *olivaceus*-group), but the differences between the groups are far more considerable than those found by Waksman and Joffe (19). The groups of *Act. griseus*, *griseoflavus*, *cellulosae*, and *olivaceus* change the reaction only to

TABLE 7
Changes of reaction in cultures of actinomycetes on dextrose-NH₄Cl solution
Initial reaction pH 6.5

SPECIES-GROUP	STRAIN	FINAL pH	SPECIES-GROUP	STRAIN	FINAL pH
<i>Actinomyces griseus</i>	AL. VIII.	4.9-5.0	<i>Actinomyces violaceus-ruber</i>	HL. II.	4.6-4.6
	H. VI.	4.9-4.9		HL. III.	4.6-4.8
	V. III.	5.1-5.2		VB. III.	4.6-4.6
	W. aut.	4.9-4.9		W. aut.	4.4-4.5
<i>Actinomyces griseoflavus</i>	Hed. IV.	4.9-4.9	<i>Actinomyces roseus</i>	B. V.	3.7-3.8
	A. XVIII.	4.9-4.9		V. II.	3.6-3.7
<i>Actinomyces cellulosae</i>	Sp. I.	4.8-4.9	<i>Actinomyces bobili</i>	V. XII.	3.8-3.9
	A. V.	4.9-4.9		V. XIII.	3.7-3.8
	AL. III.	4.8-4.9		W. aut.	3.9-3.9 ^{sp}
<i>Actinomyces olivaceus</i>	Sp. III.	5.0-5.1	<i>Actinomyces Halstedii</i> (?)	B. IX.	3.0-3.1
	L. II.	5.4-5.5		B. XIX.	3.4-3.5
	SD.	4.8-4.8	<i>Actinomyces viridochromogenus</i>	SVI (?)	4.1-4.2
	W. aut.	4.8-5.0		W. aut.	4.2-4.2
<i>Actinomyces fulvisimus</i>	H. I.	4.2-4.2	<i>Actinomyces aureus</i> (?)	LS.	3.9-4.1
	T. XIV.	4.2-4.2		Hb.	4.6-4.7
	R. V.	4.2-4.3	<i>Actinomyces pheochromogenus</i>		
	V. XXII	4.3-4.3			
<i>Actinomyces erythrochromogenus</i>	T. IV.	4.1-4.2			

TABLE 8
Growth of actinomycetes at pH 7.40 and 4.55

ORGANISM	GROWTH AT	
	pH 7.40	pH 4.55
<i>Act. griseus</i> , 3 strains.....	4-5	0
<i>Act. griseoflavus</i> , 2 strains.....	5	0
<i>Act. cellulosae</i> , 3 strains.....	4-5	0
<i>Act. olivaceus</i> , 3 strains.....	4-5	0
<i>Act. fulvisimus</i> , 3 strains.....	3-4	5
<i>Act. violaceus-ruber</i> , 3 strains.....	3-5	0
<i>Act. roseus</i> , 2 strains.....	2-3	3-4
<i>Act. bobili</i> , 2 strains.....	3-4	2-4
<i>Act. Halstedii</i> (?), 2 strains.....	4-5	4-5
<i>Act. aureus</i> (?).....	4-5	4-5
<i>Act. erythrochromogenus</i>	3-3	3-4

pH 4.8 to 5.1—just the value which was found critical for the actinomycetes studied by Waksman and Joffe, and by Gillespie. *Act. violaceus-ruber* goes a little further, to pH 4.4 to 4.6, and the rest of the strains are able to resist still higher concentrations of hydrogen ions, one strain of *Act. Halstedii* (even acidifying the medium to pH 3. As a control on these figures a small experiment was run on the ability of the actinomycetes to induce growth at acid reaction. The following solution was used: starch, 10 gm.; asparagin, 2 gm.; K_2HPO_4 , 2 gm.; $MgSO_4$, 0.5 gm.; NaCl, 0.5 gm.; distilled water, 1,000 cc. Since lack of time prevented the carrying out of an experiment over a wide range of pH values, only the original medium of pH 7.4 and a medium with reaction adjusted to pH 4.55 by means of hydrochloric acid were used. The arrangement of the experiment was as before. After 10 days' growth at 25°C. the results found in table 8 were noted.

They agree fully with the previous experiment: the groups which acidify the medium only to pH 4.8 to 5.0 all refuse to grow at pH 4.55, as does *Act. violaceus-ruber*, for which the pH region of 4.4 to 4.6 is just the critical value; but all the more resistant strains produce a more or less vigorous growth at this reaction. The limiting pH value thus seems to be of considerable value for characterizing the various species-groups.

CONCLUSIONS

As was mentioned in the preface, all investigators agree about the great variability of the actinomycetes, which may vary in their appearance, not only on different media at the same time, but also on the same medium at different periods of time, so much that it is hardly ever possible to obtain two strains that show entire agreement. However, as Waksman (11) points out, closer study will often show these variations to be quantitative rather than qualitative, so that it is possible to classify them in groups, the limits of which must still be rather vague, since they must generally be left to the judgment of each individual investigator. In the present work this great variability was fully recognized, but in spite of this, certain outstanding features remain, which may serve for characterization of the various species-groups. For instance, all the strains of the *griseus* and *griseoflavus* groups have their strong proteolytic power, especially characterized by an abundant ammonia formation; this was found true of freshly isolated strains (H. IV, V. III) as well as of strains which had been kept in culture for more than two years (A. L. VIII, A. XVIII, Hed. IV); also the characteristic colors of the aerial mycelia remained constant. Further, the pigment production, which generally is subject to immense variation, may in several cases prove a qualitatively constant feature; for instance, none of the "chromogenus" species were ever seen to lose their property of forming brown pigments in protein media during the three years over which the work was extended, and the strains of the *fulvissimus* group retained their formation of the typical

golden pigment during the same period, although the intensity and brightness of the color varied considerably on the different media. Also such features as the litmus-like pigment and the strong nitrate reduction in the *violaceus-ruber* group and the peculiar appearance of the vegetative mycelium in the *bobili* group proved most characteristic. Simply composed, synthetic media are certainly the most suitable for bringing out the characteristic features in the vast majority of the cases [it is quite possible that Lieske's (9) conviction of the impossibility of classifying the actinomycetes is due to the fact that he did not employ any such media], but instances may also be found, where complex organic media will give a characteristic growth, such as the very striking appearance of the *bobili* group on nutrient agar with glycerin. Finally the resistance towards acidity promises to be of value for separating the groups from one another and should be tested on a much larger number of strains than has been possible here. Although the majority of the strains isolated during the course of these experiments could not be identified and many of them not classified in groups, the author feels that this failure is due more to a lack of a sufficient number of representatives for the various groups and to a lack of sufficiently thorough study of both morphological and physiological characters than to an inherent impossibility of classifying these organisms.

SUMMARY

Counts were made of actinomycetes in 56 Danish soils of different character. The numbers arranged from none or a few thousands to about 13 millions to a gram of soil. Nearly all strongly acid soils—of pH less than 5—had very small numbers of actinomycetes. The highest numbers seemed most frequent in the pH interval of 6.8 to 8. The percentage of actinomycetes in the total microflora on plates varied from 0 to 73 per cent; this figure was very low in strongly acid peat soils; otherwise it did not show any correlation with the soil reaction. Apart from the reaction there seemed to be no correspondence between the general soil types and the numbers of actinomycetes.

Several strains of actinomycetes isolated from these soils could be recognized as belonging to the following species-groups:

Common organisms: *Act. griseus*, *cellulosae*, *olivaceus*, *bobili*, *diastatochromogenus*. Fairly common to rare organisms: *Act. griseoflavus*, *violaceus-ruber*, *roseus*, *erythrochromogenus*, *pheochromogenus*. A new species-group, *Actinomyces fulvissimus* n. sp., is described.

The majority of the strains could not be identified as belonging to definite groups. The different strains within the same species-group often show great differences in their physiological characters, such as pigment production, ability to utilize various carbohydrates, acid formation, nitrate reduction, and proteolytic activity although some of these characters remained relatively constant and appeared to have diagnostic value. Organic acids were some-

times formed from carbohydrates; this was always connected with nitrite production. The "chromogenous" forms had, upon the whole, a lower proteolytic power than the non-chromogenous. The ability of actinomycetes to resist acid reaction varied widely; the final acidity produced in a physiologically acid nutrient solution for each species-group was subject to less variation than most other physiological characters and may be of value for the characterization and identification of the different species-groups.

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EXCHANGEABLE CATIONS AND LIME REQUIREMENT IN DIFFERENTLY FERTILIZED SOILS¹

S. RAVIKOVITCH²

New Jersey Agricultural Experiment Station

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The application of different fertilizers to the soil can be considered as an important factor affecting the amounts and kind of cases in the soil exchange complex. The soluble fertilizers introduced into the soil, change the concentration of the soil solution, which is established as a result of equilibrium reached between the dissolved salts of the soil and the exchangeable cations of the exchange complex. As a consequence of the change in the concentration of the soil solution, an exchange reaction between the cations of the salt introduced and the cations of the exchange complex occurs. A part of the cations of the salts is absorbed, and an equivalent amount of the exchangeable cations appears in the solution.

The amount of the bases in the complex can be increased or decreased, depending on the nature of the fertilizers applied to the soil. Hissink (10) points out the effect of the so-called acid and alkaline fertilizers on the exchangeable calcium in soils. The acid fertilizer is found to bring a marked decrease in the content of the exchangeable bases, as compared with the effect of the alkaline fertilizers. Page and Williams (25) emphasize the influence of different fertilizers on the proportion and content of the exchangeable bases in the Rothamsted soils. The results were obtained from fertilized plots which were chalked. By applications of gypsum and of potassium and magnesium salts, the cations of these salts were introduced into the exchange complex. The addition of chalk to the soils increased the amount of exchangeable calcium. Merkle (23) found that fertilizers and organic manure change the quantity and proportion of the exchangeable cations. Ammonium and potassium when applied as fertilizers are retained by the acidoid complex.

The introduction of the different fertilizers to the soil over a period of years is expected to exert a sensible effect on the soil exchange complex; this effect

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being more marked in soils which are low in soluble salts and have a low base exchange capacity.

Observations on the effect produced by fertilizers on the state of the soil, are mostly related to certain chemical changes, especially those in the soil reaction. Few investigators have studied the effect of the different fertilizers on the kind and amount of exchangeable bases of soils.

The present work was performed to study the effect produced by different fertilizers in a Sassafras loam soil in 20 years of fertilization on the content of the exchangeable calcium and magnesium in the soil, on the degree of unsaturation, and on the lime requirement and reaction. This work gave an opportunity to study the effect produced by the fertilizers in the presence and absence of lime in soil.

SOILS USED

Samples of soil taken at a depth of $6\frac{1}{2}$ inches from differently fertilized plots of the New Jersey Agricultural Experiment Station were air dried and passed through a 1-mm. sieve for this study. The soil is a Sassafras loam and has a low exchange capacity—4.50 and 5.69 milliequivalents of bases to 100 gm of soil for the unlimed and limed soils of the check plots respectively. The ratio of silica to sesquioxides of the colloidal part of an adjacent virgin Sassafras soil is 1.66. The sandy character of this soil and the low ratio of $\frac{\text{SiO}_2}{\text{Al}_2\text{O}_3 + \text{Fe}_2\text{O}_3}$

explain the fact of the low base exchange capacity of this soil. The silica sesquioxide ratio of the colloids is found by Mattson (20) to be directly related to their property to adsorb bases. This investigator observed that with the increase of the ratio of the silica to sesquioxides, there is an increase in the capacity of adsorption by the colloid.

The experimental plots were established in 1908 by Lipman and Blair (13) for the study of the availability of the different nitrogenous fertilizers. The plots receiving different treatments are divided into two sections: A and B. Plots A have received no lime for the last 20 years, whereas plots B has received lime at intervals of 5 years (2 tons ground limestone to the acre). The fertilizer treatment is the same for the corresponding plots in each series (13, 14, 15, 16). Eighteen plots were selected for the experiments, nine from the unlimed section A and nine from the corresponding limed section B. The fertilizer treatments of the plots selected were as follows:

<i>Plot No.</i>	<i>Annual fertilizer treatment for 1/20-acre plot</i>
1A, 1B	Nothing
2A, 2B	16 pounds muriate of potash
3A, 3B	32 pounds superphosphate
4A, 4B	Minerals only*

* Minerals—32 pounds superphosphate and 16 pounds muriate of potash. (Since 1923, some changes have been made in the application of superphosphate and muriate of potash, which were reduced to one-half of the original amount, that is, 16 and 8 pounds respectively to each plot.)

<i>Plot No.</i>	<i>Annual fertilizer treatment for 1/20-acre plot</i>
5A, 5B	Minerals, 1600 pounds cow manure
9A, 9B	Minerals, 16 pounds NaNO_3
10A, 10B	Minerals, $\text{Ca}(\text{NO}_3)_2$ equivalent to 16 pounds NaNO_3
11A, 11B	Minerals, $(\text{NH}_4)_2\text{SO}_4$ equivalent to 16 pounds NaNO_3
12A, 12B	Minerals, CaCN_2 equivalent to 16 pounds NaNO_3

METHODS

In performing this work, difficulties were met with in applying some of the methods; therefore, more detailed study was necessary. This paper includes the results of the study and a comparison of certain methods which were used afterward in the carrying out of this research.

To determine the amount of the exchangeable calcium in the unlimed soils A, Gedroiz's (7) method was used. Twenty-five gram samples were treated with a neutral normal solution of ammonium chloride. Half a liter of this salt solution was found to be sufficient for complete replacement of the exchangeable calcium in the soil.

In the limed soils B, the determination of the exchangeable calcium was more difficult. Hissink's method (9) was applied. This method consists of leaching a 25-gm. sample of soil with a neutral normal solution of sodium chloride. Hissink assumes that the first portion of the filtrate, equal to 1 liter, contains both the exchangeable calcium and the calcium carbonate brought into the solution by the action of the sodium chloride, and that the second liter contains the dissolved calcium carbonate, equal in amount to that of the first liter. The difference between the amounts of calcium in the first and second liters of the filtrate represents the amount of the exchangeable calcium present in the soil.

In the case of the Sassafras B soils, with a low base exchange capacity, 12.5-gm. samples were treated with sodium chloride solution and two half-liter portions of filtrates were collected and the calcium was determined. By subtracting the amount of calcium found in the second portion of the filtrate from that in the first portion, it appeared that the amounts of exchangeable calcium were too high. The sum of the exchangeable calcium and magnesium in most cases surpassed the total base exchange capacity of the soils tested, even when sodium, potassium, and hydrogen originally present in the soil exchange complex were not taken into consideration. The cause of this phenomenon was supposed to be due to the unequal solubility effect produced by the sodium chloride solution on the calcium carbonate of the soil in the first half-liter treatment and in the subsequent second half-liter treatment, the solubility being higher in the former case.

Two other methods, those of Tyurin (26) and Gedroiz (7) were then applied for determining the amount of the dissolved calcium carbonate in the first half liter of the sodium chloride solution. Tyurin recently suggested, for ascertaining the dissolved carbonates, a method which consists in titrating a portion of the filtrate containing both the exchangeable and the dissolved cal-

cium carbonate with a 0.02 *N* solution of hydrochloric acid in the presence of methyl orange. From the amount of hydrochloric acid used for the decomposition of the carbonates present in the solution, the sum of calcium oxide and magnesium oxide is calculated in terms of calcium oxide, because of the predominance of calcium carbonate in the salt solution. Determinations of the dissolved calcium carbonate were made by this method. The absence of magnesium carbonate or the presence of only traces permitted the calculation in equivalents of calcium from the results obtained by titration.

Gedroiz's method for determining the dissolved calcium carbonate consists in estimating the carbonates in the soil before and after treatment with the salt solution. The difference between the carbonates found before and after treatment gives the amount of the carbonates dissolved.

TABLE 1

Amount of calcium carbonate dissolved from the soils by the normal sodium chloride solution, as determined by different methods

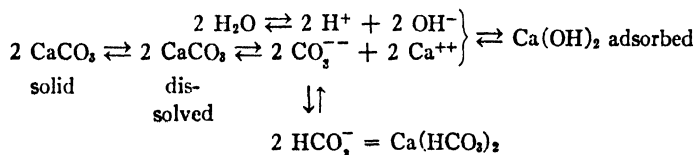
PLOT NO.	CaO DISSOLVED FROM 12.5 CM. OF SOIL			CaCO ₂ PRESENT IN THE SOIL
	Titration method (Tyurin)	Difference in content of CaCO ₃ before and after treatment with <i>N</i> NaCl solution (Gedroiz)	Ca found in the second half liter of NaCl solution (Hissink)	
	mgm.	mgm.	mgm.	per cent
1B	5.2	5.2	2.4	0.28
2B	3.1	3.6	0.7	0.06
3B	4.5	6.0	1.5	0.16
4B	4.3	4.1	1.0	0.09
5B	4.5	4.6	1.4	0.05
9B	3.4	3.5	0.8	0.06
10B	4.5	4.6	1.4	0.13
11B	3.2	3.8	0.8	0.08
12B	4.6	5.0	1.1	0.08

The amounts of calcium carbonate dissolved, as determined by Hissink's, Tyurin's, and Gedroiz's methods, are presented in table 1. A comparison of the results shows that according to Tyurin's method the amounts of the calcium carbonates dissolved are almost similar to those obtained by Gedroiz's method. In the case of Hissink's method, the results are found to be much lower. By introducing a correction for the dissolved calcium carbonate according to the Hissink method, the amount of the exchangeable calcium is thus found to be too large. Hissink's method gives values for the exchangeable calcium which are greater than those of the two other methods by 0.68 to 1.33 milliequivalent to 100 gm. of soil for the different plots. These amounts introduce a fairly considerable error in soils with a low exchange capacity such as Sassafras loam.

Menchikowsky and Ravikovitch (22) used the method of Hissink for the determination of the exchangeable calcium in highly calcareous soils (15.5 to

40.1 per cent CaCO_3). The results obtained showed that the second liter of sodium chloride solution contained almost a constant concentration of calcium carbonate dissolved for the different soils examined. For the Sassafras soils B, with a low content of calcium carbonate (table 1), the constancy of concentration of the calcium carbonate dissolved was not observed. This constancy of solubility produced by the sodium chloride solution may be expected in soils with a high content of calcium carbonate.

The exchangeable hydrogen (unsaturation) was determined by Mattson's calcium carbonate paste method (21), which is based on the principle of interaction between an unsaturated soil and calcium carbonate paste through a parchment membrane. As a result of this contact with the paste, the hydrogen of the complex is replaced by the calcium. Mattson (18) observed that when calcium carbonate is mixed with a soil in the presence of water, as a result of the hydrolysis of the calcium carbonate an amount of calcium is adsorbed by the soil and an equivalent amount of calcium is left in the solution as calcium bicarbonate. The reaction of hydrolysis of calcium carbonate is shown in the following scheme:



The calcium hydroxide formed as a result of hydrolysis of the calcium carbonate, is adsorbed by the soil until it is in equilibrium.

The 12.5-gm. samples of soils to be tested were placed in conical bags of thin parchment paper, which were then put into similar shaped bags made of filter paper. Then bags containing the soil were placed in a calcium carbonate paste. The soils were then saturated with water and allowed to remain in the paste until equilibrium between the calcium carbonate and the soil was established. The filter paper bag was introduced throughout this work to keep the parchment bag from direct contact with the solid calcium carbonate and thus to prevent it from sticking to the parchment bags. After equilibrium was established, the inner parchment bag containing the soil was then removed for analysis.

A more detailed study of this method established the fact that during the first period of contact of the soil with the paste, the samples tested contained amounts of calcium (as determined by treatment with ammonium chloride and sodium chloride solutions) sometimes higher than the total base exchange capacity. This rise in calcium content is due to the formation in the soil mass of calcium bicarbonate, which appears simultaneously with the calcium hydroxide. As a result of the intensive adsorption of the calcium hydroxide by the soil in the early stages, the calcium bicarbonate accumulates temporarily in the soil solution. The accumulation of the bicarbonates on the inside of

the membrane produces a diffusion gradient toward the outside paste solution which has a lower concentration of this salt and a higher pH, and thus the calcium bicarbonate is gradually removed from the soil.

The preliminary experiments showed that a period of 21 days is necessary for complete saturation of the soils and that a period of 27 days is sufficient for complete removal of the excess calcium bicarbonate from the soil, as equilibrium is already established. By leaching the samples with water before treatment with the salt solutions, the calcium bicarbonate present in the soil mass after the saturation is completed, can be removed. The results obtained for the amount of calcium after 27 days of contact with the paste, and the same

TABLE 2
Amount of exchangeable bases in the unlimed and limed soils

UNLIMED				LIMED						
Plot no.	CaO		Ca, in per cent of the total base exchange capacity	Plot no.	CaO		Ca, in per cent of the total base exchange capacity	MgO		Mg, in per cent of the total base exchange capacity
	Per cent	Milliequivalents in 100 gm. soil			Per cent	Milliequivalents in 100 gm. soil		Per cent	Milliequivalents in 100 gm. soil	
1A	0.057	2.05	45.5	1B	0.141	5.03	88.4	0.007	0.36	6.3
2A	0.039	1.40	30.4	2B	0.112	3.98	72.4	0.008	0.40	7.3
3A	0.074	2.65	50.2	3B	0.136	4.84	80.3	0.006	0.32	5.3
4A	0.063	2.25	46.0	4B	0.144	5.15	80.5	0.005	0.24	3.8
5A	0.075	2.65	48.2	5B	0.190	6.77	80.7	0.020	0.95	11.3
9A	0.075	2.65	51.1	9B	0.126	4.48	78.1	0.009	0.44	7.5
10A	0.074	2.63	52.3	10B	0.136	4.86	82.2	0.009	0.44	7.4
11A	0.011	0.40	8.7	11B	0.109	3.90	68.7	0.008	0.40	7.0
12A	0.075	2.66	59.9	12B	0.143	5.09	78.4	0.016	0.78	12.0

soils after 21 days of contact when washed before treatment with the salt solution, were found to be identical.

After equilibrium was reached, the A soil samples were treated with ammonium chloride solution and the B soils with sodium chloride solution. In the filtrate the calcium, which was determined, represents the sum of the exchangeable and the adsorbed calcium, and in the case of the limed B soils, also the dissolved calcium carbonate. The difference between the amount of calcium before and after contact with the paste is the calcium adsorbed, which, expressed in milliequivalents to 100 gm. of soil, represents the H-ion content in the soil exchange complex.

The total base exchange capacity was determined by treating the soil with a neutral normal solution of barium chloride until the cations present in the complex were replaced by barium ions.

The hydrogen-ion concentration of the soils examined was determined potentiometrically by the quinhydrone electrode.

The determinations of silicon, aluminum, and ferric oxides in the colloidal part of the Sassafras soil were made by fusion with sodium carbonate (2).

All calculations are made on the basis of oven-dry matter.

EXCHANGEABLE BASES IN THE SOILS

Influence of lime on the exchangeable calcium content

The amounts of exchangeable calcium for plots A and exchangeable calcium and magnesium for plots B are given in table 2 and presented graphically in figure 1.

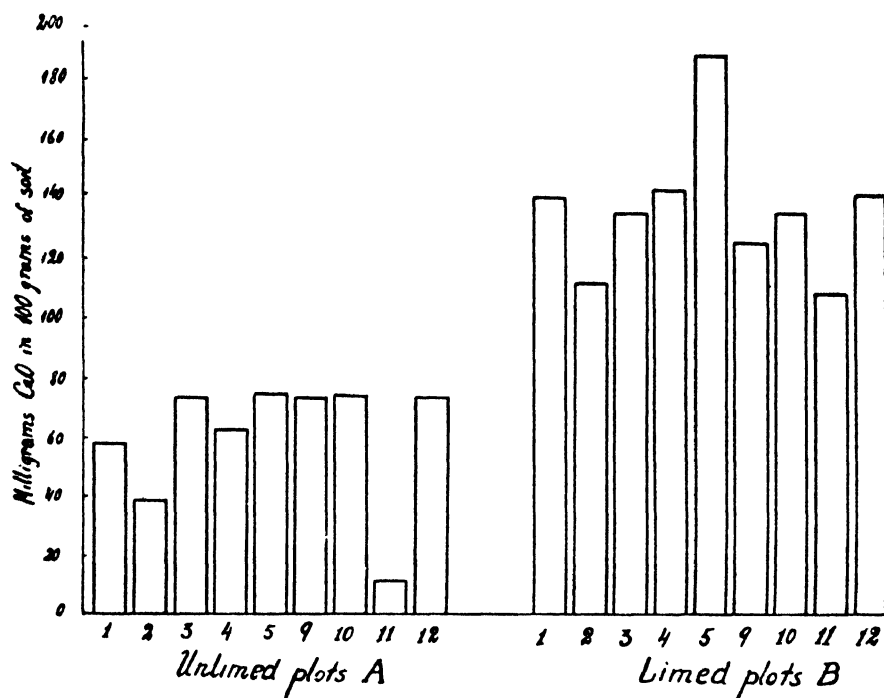


FIG. 1. AMOUNT OF EXCHANGEABLE CALCIUM IN UNLIMED AND LIMED SOILS

From the results obtained, the difference between the amounts of the exchangeable calcium in plots A and B is readily seen. The calcium content of plots B considerably exceeds that of plots A. The increase in the exchangeable calcium content in the B plots is due to the introduction of lime to these soils. Thus this marked difference explains clearly the influence of the liming on the content of exchangeable calcium in the soils. Table 3 shows the effect of liming (CaCO_3) upon the amounts of exchangeable calcium in the soils. The increase in the percentage of exchangeable calcium in the B plots, due to the liming, varies from 40.6 per cent to 89.7 per cent.

Influence of fertilizers on exchangeable calcium content

A comparison of the amounts of the exchangeable calcium for the different plots in each of the two series A and B, shows a difference in their content, which is due to the influence of the different fertilizers.

In series A, the exchangeable calcium is lowest in plots 11, 2, and 1. The remainder of the plots, with the exception of 4A, contain equal amounts of exchangeable calcium (2.63 to 2.68 milliequivalents to 100 gm. of soil).

Plot 2A, receiving the muriate of potash, lost a considerable part of its exchangeable calcium. Plot 4A, receiving calcium phosphate, in addition to muriate of potash, contains a higher amount of exchangeable calcium than plot 2A, receiving only muriate of potash. The calcium phosphate, with its constituent calcium sulfate, tends to increase the amount of the exchangeable

TABLE 3
Effect of liming upon the amount of exchangeable calcium in the soils

PLOT NO.	DIFFERENCE IN EXCHANGEABLE CALCIUM BETWEEN THE LIMED AND UNLIMED SOILS		INCREASE OF EXCHANGE CALCIUM IN LIMED PLOTS, DUE TO LIMING
	Per cent CaO	Milliequivalents CaO to 100 gm. soil	
			<i>per cent</i>
1B	0.084	2.98	59.2
2B	0.073	2.58	64.8
3B	0.062	2.19	45.2
4B	0.081	2.90	56.3
5B	0.115	4.09	60.4
9B	0.051	1.82	40.6
10B	0.062	2.23	45.9
11B	0.098	3.50	89.7
12B	0.068	2.43	47.5

calcium in the soil, and, in mixture with the muriate of potash, suppresses the replacing effect of the latter on the exchangeable calcium.

Plot 5A, 9A, 10A, and 12A, receiving in addition to muriate of potash and superphosphate, different nitrogenous fertilizers, such as cow manure, sodium nitrate, calcium nitrate, and calcium cyanamid, respectively, are about equal in their content of exchangeable calcium. These different nitrogenous fertilizers did not induce any comparative changes in the content of exchangeable calcium. The nearly constant amount of the exchangeable calcium in these plots can be related to a balance between the augmenting and suppressing effect of the superphosphate and muriate of potash respectively.

Calcium phosphate and muriate of potash, brought into soils A, create in the solution a definite concentration of these salts. The exchangeable calcium is thus maintained at an equilibrium with the soil solution, and is held at a certain level, which is regulated by the concentration of the calcium and potassium ions of the salts applied. When the exchangeable complex is subjected

to the action of only the potassium ion of the muriate of potash, as in the case of the plot 2A, the effect of this ion is expected to be different from that of the calcium ion of the superphosphate. In this plot the potassium, not having the competition of calcium, exerted its action by removing the calcium from the exchangeable complex. The continual introduction of the muriate of potash into the soil in the course of 20 years, at the rate of 320 or 160 pounds an acre, reduced the amount of the calcium over 30 per cent, as compared with the check plot 1A.

A very considerable change in the content of the exchangeable calcium in the soil was produced by the application of the ammonium sulfate. This salt reduced the amount of the exchangeable calcium to the very low level of 0.4 milligram equivalent to 100 gm. of soil. As a physiological acid salt, the ammonium sulfate brings into the soil two acid radicals, nitrate and sulfate (the first appearing as the result of nitrification). The acids formed, continuously attack the soil exchange complex and remove the combined bases as salts of the nitric and sulfuric acids. The annual introduction of new portions of this salt to the soil, decidedly exhausted the soil of its exchangeable calcium content, which is 85 per cent lower than the plots receiving other forms of nitrogenous fertilizers.

The comparatively lower content of the exchangeable calcium in the check plot 1A can be explained by the fact that this plot did not receive, as did the other plots, calcium phosphate, which leads to a certain increase of the exchangeable calcium.

The exchangeable calcium content of the limed plots of series B varies. In those plots the effect of muriate of potash and of ammonium sulfate in decreasing the content of the exchangeable calcium can also be observed. This influence is less pronounced than in the unlimed plots 2A and 11A on account of the presence of calcium carbonate, which maintains the saturation of the soil.

Plot 5B, receiving cow manure, surmounts the other plots in its content of exchangeable calcium. This high content is related to the high exchange capacity of the soil. The higher capacity is due to the application of organic manure, which introduces into the soil certain organic compounds possessing the property of base exchange in a very high degree.

The other limed plots are almost equal in their amounts of exchangeable calcium.

In most cases, the exchangeable calcium for the different plots is found to be approximately 80 per cent of the total base exchange capacity. The relatively high percentage of exchangeable calcium in plot 1B is related to the higher content of calcium carbonate found in this soil.

Exchangeable magnesium

In table 2 are given the results for the exchangeable magnesium. The content of this base in the exchange complex varies from 3.8 per cent to 12 per

cent. The highest content of exchangeable magnesium, like that of exchangeable calcium is in plot 5B.

Calcium carbonate in the limed plots

In the last column of table 1 is given the calcium carbonate content of the limed soils. The results indicate that the amounts of calcium carbonate differ for the different plots in spite of equal applications of lime to all the plots. With the exception of 10B, in all plots to which muriate of potash was applied, the content of calcium carbonate is lower than in the check plot 1B and in plot 3B, which received only superphosphate. The potassium chloride has a solubility effect not only on the exchangeable calcium, as has been pointed out, but also on carbonate of calcium. The action of muriate of potash on calcium

TABLE 4

Amount of calcium adsorbed in the unlimed and limed soils, after equilibrium in the calcium carbonate paste

UNLIMED					LIMED				
Plot no.	CaO after equilibrium in the CaCO_3 paste		CaO adsorbed		Plot no.	CaO after equilibrium in the CaCO_3 paste		CaO adsorbed	
	Per cent	Milliequiv- alents in 100 gm. soil	Per cent	Milliequiv- alents in 100 gm. soil		Per cent	Milliequiv- alents in 100 gm. soil	Per cent	Milliequiv- alents in 100 gm. soil
1A	0.110	3.92	0.052	1.87	1B	0.144	5.15	0.003	0.12
2A	0.106	3.78	0.067	2.38	2B	0.135	4.85	0.025	0.87
3A	0.139	4.96	0.065	2.31	3B	0.145	5.19	0.010	0.35
4A	0.127	4.54	0.064	2.29	4B	0.158	5.64	0.014	0.49
5A	0.137	4.88	0.062	2.20	5B	0.207	7.40	0.018	0.63
9A	0.132	4.74	0.058	2.03	9B	0.137	4.91	0.012	0.43
10A	0.134	4.77	0.060	2.14	10B	0.150	5.35	0.014	0.49
11A	0.114	4.06	0.102	3.66	11B	0.143	5.13	0.034	1.23
12A	0.122	4.36	0.048	1.70	12B	0.155	5.55	0.013	0.46

carbonate was observed by Goessman (8), who pointed out that common salt and muriate of potash decompose the carbonates of lime and magnesia of the soil, changing those carbonates from a comparatively insoluble form to a soluble salt, which is carried out from the soils in the drainage water.

UNSATURATION OF THE SOIL

The exchangeable complex of the soils is found to contain not only metallic ions, but also hydrogen ions. The soils, the exchangeable complex of which contains a certain amount of hydrogen ions, are called "unsaturated" as compared with "saturated," which yield only metallic cations in the exchange reaction (6).

The introduction of fertilizers changes markedly the state of saturation of the soil exchange complex; the amounts of the metallic ions can be increased

or reduced by application of the various fertilizers, depending on the nature of the last ones.

The soils of the unlimed A and limed B plots, which were treated for many years with different kinds of fertilizers, differ greatly in their state of saturation. The unsaturation values of the soils from plots A and B are presented in table 4. The figures represent the amounts of hydrogen ion in the exchangeable complex that were replaced by the calcium when brought into contact with calcium carbonate paste, according to the Mattson method, and thus characterizing the unsaturation of the different soils.

The highest unsaturation is found in plots A. These soils adsorbed great amounts of calcium from the paste, which approached the amounts of exchange-

TABLE 5
Degree of unsaturation in the unlimed and limed soils

UNLIMED				LIMED			
Plot no.	Total base exchange capacity in mgm. equiv. in 100 gm. soil	Unsaturation	pH	Plot no.	Total base exchange capacity in mgm. equiv. in 100 gm. soil	Unsaturation	pH
		<i>per cent</i>				<i>per cent</i>	
1A	4.50	41.5	5.03	1B	5.69	2.1	7.62
2A	4.60	51.7	5.03	2B	5.50	15.8	7.33
3A	5.28	43.6	5.04	3B	6.03	5.7	7.39
4A	4.89	46.9	5.05	4B	6.40	7.6	7.20
5A	5.56	39.5	5.12	5B	8.39	7.6	6.95
9A	5.21	39.9	5.47	9B	5.74	7.5	7.26
10A	5.03	42.3	5.37	10B	5.91	8.3	7.40
11A	4.61	79.4	3.90	11B	5.68	21.6	7.13
12A	4.44	38.3	5.57	12B	6.49	7.1	7.26

able calcium present in the soils and in some cases surpassed them. The degree of unsaturation is given in table 5 as the percentage of the exchangeable hydrogen in relation to the total base exchange capacity of the soil. The degree of unsaturation is calculated according to the following formula:

$$\frac{\text{Calcium adsorbed by 100 gm. of soil in mgm. equiv.} \times 100}{\text{Total base exchange capacity in 100 gm. of soil in mgm. equiv.}}$$

Unsaturation in the unlimed plots A

The degree of unsaturation for the A plots fluctuates from 38 per cent to 79 per cent depending upon the application of the different fertilizers. Figure 2 represents graphically the degree of unsaturation for the various A plots. The most unsaturated plots are found to be 2A and 11A. The muriate of potash and ammonium sulfate, producing the loss of exchangeable calcium in these did not substitute their basis for the calcium lost: The calcium removed is mostly found to be replaced by hydrogen.

As has been indicated, the effect of ammonium sulfate in the removal of the bases is due mostly to the action of the acids formed and the consequent introduction of hydrogen ions in the exchangeable complex. With muriate of potash, there is at first a replacement of other bases by the potassium ion and a subsequent loss of this base by replacement with hydrogen. The exchangeable complex saturated with monovalent bases is less stable when combined with these bases than in the case of divalent ones. Such a complex is more readily attacked by water, and as a result of this water action, the monovalent bases are more easily removed.

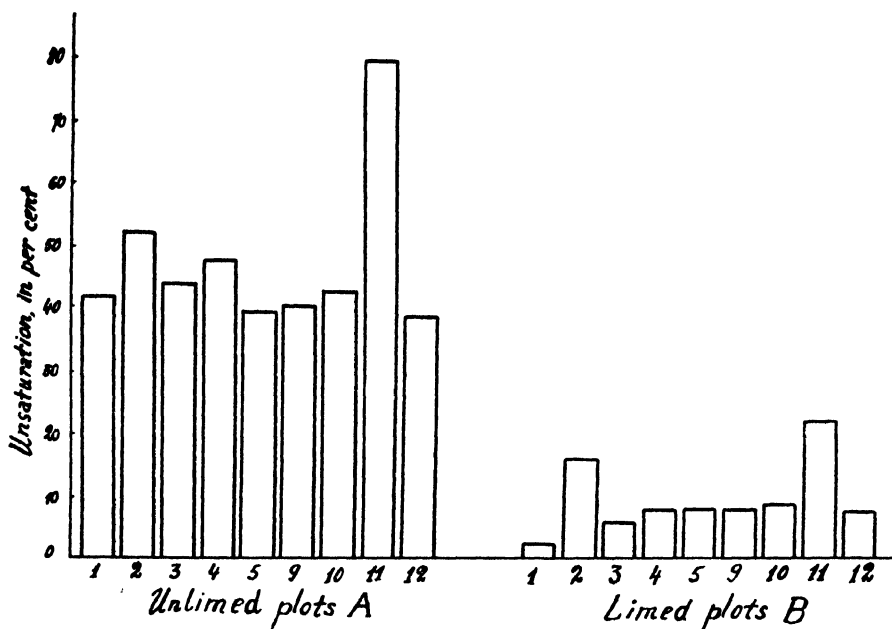


FIG. 2. DEGREE OF UNSATURATION IN THE UNLIMED AND LIMED SOILS

The exchangeable potassium can be removed also by the assimilation of plants. Martin (17) and Fraps (5), in recent papers concerning the effect of crop growth on the exchangeable potassium, show that the plants remove considerable amounts of exchangeable potassium from the soil.

Unsaturation in the limed plots B

Plots B (table 4 and fig. 2) likewise were found unsaturated to a certain degree. It can be noted that in spite of the presence of calcium carbonate in the soil all the plots contain a certain amount of hydrogen in the exchangeable complex. The presence of the replaceable hydrogen in the limed plot 11B, was previously observed by Joffe and McLean (2).

Because of many factors (such as the percolation of water through the soil,

bacterial activities, growth of plants, the effect of fertilizers), the complete saturation is evidently not reached under field conditions in the presence of small amounts of calcium carbonate. The greatest unsaturation is found in plots 2B and 11B, which is 15.8 per cent and 21.6 per cent, respectively. The unsaturation for the other B plots, with the exception of the check plot 1B, varies only between 5.7 per cent and 8.3 per cent.

LIME REQUIREMENT OF THE SOILS

In these experiments, the "lime requirement" means the amount of calcium adsorbed in the presence of unlimited quantities of calcium carbonate. This amount was determined, according to the Mattson method, by bringing the unsaturated sample of soil into contact with the calcium carbonate paste until equilibrium between the calcium carbonate and the soil was established. This

TABLE 6

"Lime requirement" (calcium oxide) for the unlimed and limed soils, according to Mattson's method

PLOT NO.	LIME REQUIREMENT (CaO) POUNDS AN ACRE	PLOT NO.	LIME REQUIREMENT (CaO) POUNDS AN ACRE
1A	1,040	1B	60
2A	1,340	2B	500
3A	1,300	3B	200
4A	1,280	4B	280
5A	1,240	5B	360
9A	1,160	9B	240
10A	1,200	10B	280
11A	2,040	11B	680
12A	960	12B	260

means of determining the lime requirement approaches more closely the natural conditions of lime application.

The rapidity and completeness of saturation will be dependent on the concentration of calcium hydroxide in the soil solution which appears as the result of the hydrolysis of the calcium carbonate. The liming under field conditions will approach the results of the laboratory experiments, and a more rapid and complete saturation of the exchangeable complex will depend on the introduction of somewhat increased amounts of lime, based on the amount found necessary by the laboratory paste method; the fineness of the lime applied; and the careful mechanical mixing of the soil after the introduction of lime.

Table 6 gives the amounts of lime, as calculated by Mattson's method, necessary for complete saturation of the soil of plots A and B. The figures are given for an acre, to a depth of $6\frac{3}{4}$ inches, on the basis of 2,000,000 pounds of soil.

COMPARISON OF LIME REQUIREMENT AND pH VALUE IN THE SOILS

Many experiments were carried out to compare the lime requirement with the pH value in an attempt to establish a correlation between the two values. Often, soils having the same pH values are found to vary in their lime requirement. Christensen and Jensen (4), investigating a number of soils having the same pH value, found a great difference in the amount of lime required for neutralization. Johnson (11) points out that there is no relation between the lime requirement and the hydrogen-ion concentration. Joffe and McLean (12) showed no correlation to exist between the pH value and the total replaceable hydrogen in the complex. Other investigators, however, found a correlation

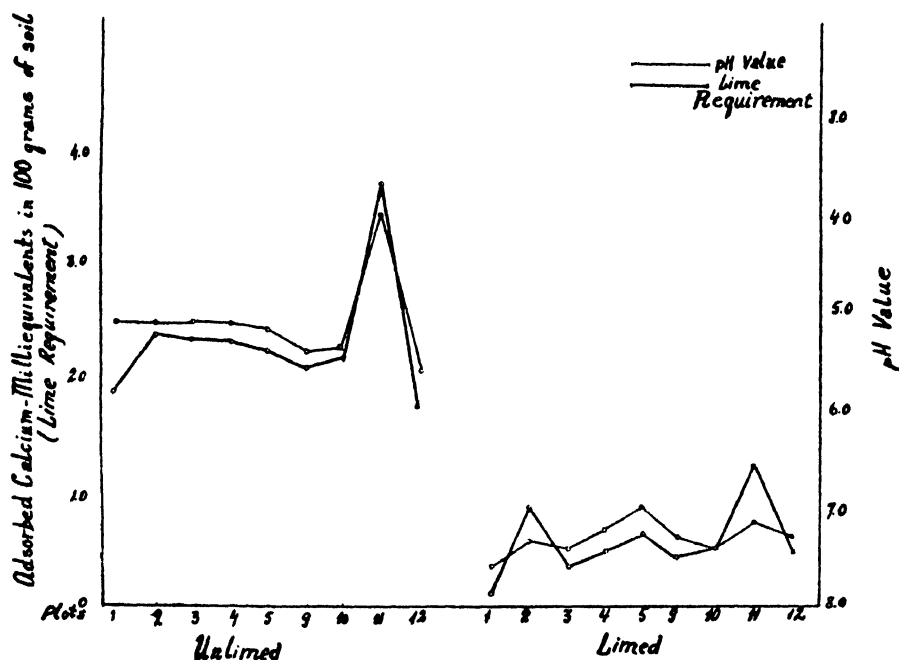


FIG. 3. RELATIONSHIP BETWEEN THE "LIME REQUIREMENT" AND THE pH VALUES

between the two values. Blair and Prince (2) found a fairly close correlation between the H-ion concentration and the lime requirement. Some correlation has been obtained by Carleton (3) for the hydrogen-ion concentration and lime requirement for soils of the same type.

Ogg and Dow (24) showed that heavy soils had a higher lime requirement than light soils.

The relationship between the two values depends chiefly on the buffer and adsorption capacity of the different types of soil. Mattson (19, 20) showed that the colloidal material of different soils varies greatly in base adsorption power according to their composition ratio of silica/sesquioxides. The buffer and exchange capacity increases with an increase in this ratio.

In the case of the Sassafras soils with different lime requirements for various plots which belong to the same type of soil and have almost the same physical and chemical composition for each section, an opportunity was given to find whether a relationship exists between the lime requirement and the pH value. The results obtained (tables 5 and 6) for the lime requirement and for the reaction of all the unlimed and limed plots show that there is a certain correlation between these two values. The reaction of the plots unsaturated to different degrees thus reflects the state of their unsaturation. The correlation is graphically represented in figure 3.

The reaction of the soils

Table 6 gives the pH value of the unlimed A and limed B plots. The pH of the A plots varies from 3.9 to 5.57. The pH for all the limed plots is higher, the reaction being alkaline in all plots with the exception of 5B. Plots 2B and 11B, of which the degree of unsaturation rises to 15.8 and 21.6 per cent respectively, are slightly alkaline. Thus it is seen that the soils are unsaturated with respect to their reaction with calcium carbonate and still have an alkaline reaction. The calcium-magnesium-sodium-potassium-hydrogen complex is analogous to the salts of the sodium phosphate, which may contain displaceable hydrogen and not be acid. Similarly, the reaction of a soil may be alkaline and yet react with calcium carbonate.

From the foregoing discussion it is seen that the reaction reflects relatively the state of unsaturation of the soil studied, but does not show the actual degree of base saturation of the exchange complex.

SUMMARY

Some of the methods applied were studied in detail and discussed. Mattson's calcium carbonate paste method was studied in detail before its application to the determination of lime requirement. Hissink's, Gedroiz's, and Tyurin's methods for the determination of the calcium carbonate dissolved by the sodium chloride solution, were compared. Hissink's correction for the dissolved calcium carbonate was found to be unsatisfactory for the limed Sassafras soils. Gedroiz's and Tyurin's methods were found to give almost identical results for the amount of calcium carbonate dissolved.

The effect of different fertilizer treatments on the content of exchangeable calcium and magnesium, the degree of unsaturation, the lime requirement, and the reaction were studied. The amount of exchangeable calcium is found to vary with the kind of fertilizer applied to the soil. The calcium carbonate in all cases increased considerably the amount of exchangeable calcium. The superphosphate increased to a certain degree the amount of the exchangeable calcium. The muriate of potash is found to remove the exchangeable calcium both in the unlimed and limed soils. The ammonium sulfate appeared to have the most exhaustive action in removing the exchangeable calcium from the soil. The greatest effect was produced on the unlimed soil. The application of manure increased the total base exchange capacity of the soils and in the

limed plot, the amount of the exchangeable calcium. The unlimed soils were found to be highly unsaturated, the degree of unsaturation being influenced by the fertilizer applied. A correlation was found between the lime requirement and the pH value, in both the unlimed and the limed soils.

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A CRITICAL STUDY OF THE METHODS FOR DETERMINING THE NATURE AND ABUNDANCE OF SOIL ORGANIC MATTER¹

SELMAN A. WAKSMAN AND KENNETH R. STEVENS

New Jersey Agricultural Experiment Station

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Among the numerous problems dealing with soil organic matter, which is complicated both in nature and origin, none has received more attention than the methods suggested for its quantitative determination. These methods have been concerned either with the determination of the soil organic matter as a whole or only with a certain definite fraction that has been commonly considered to be of the greatest importance in soil fertility. The term "humus" is usually applied indiscriminately both to the total organic matter and to some portion of it which has become an integral part of the soil.

METHODS FOR DETERMINATING TOTAL SOIL ORGANIC MATTER

It is now generally agreed that the determination of the total organic matter content of the soil by the loss on ignition is hardly a fair measure, since the chemically combined water is also included in this determination. The common error found in the use of this method may range from 10 per cent, in the case of sandy soils, to 50 or even 70 per cent, in the case of clay soils. This unusually large error makes the determination totally unacceptable for accurate work.

The other common method available for this purpose is the determination of the total carbon content of the soil; this can be obtained by one of the dry or one of the wet combustion methods. The amount of organic carbon found in a definite quantity of soil is then multiplied by the factor 1.724 to give the total content of soil organic matter. This factor has been obtained by Van Bemmelen, Wollny, and other soil chemists, on the assumption that the carbon content of soil organic matter is 58 per cent. It will be brought out in the following pages that this assumption was fairly accurate and that this method is still the most reliable that we have at the present time for measuring the total organic matter of the soil. If the soil contains carbonates, these should be determined separately and correction made on the total carbon found in the given soil.

Because the carbon-nitrogen ratio of the soil organic matter is usually found to be about 10 to 1, it has been suggested that the organic matter content of

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the soil be calculated from the total nitrogen found (1), namely, by multiplying the nitrogen figure by 20. This is often far from accurate, however, because the ratio (C:N) is frequently found to be much narrower than 10:1, in which case an abnormally high organic matter content will be reported.

Various modifications of the total carbon method have been suggested, based upon the oxidation of the organic matter, whereby either the amount of oxidizing agent consumed, as in the chromic acid method of Schollenberger (13), or the amount of reduction brought about, as in the sulfur dioxide method of Robinson (9), is determined. These methods may prove convenient for practical purposes where a rapid determination of the approximate concentration of organic matter in the soil is desired. The results will be invalidated, however, when the soil is rich in reducing substances of an inorganic nature. The use of a 30 per cent solution of hydrogen peroxide for the complete oxidation of the total soil organic matter can also be grouped among the methods for determining total carbon in soil (10).

For a careful qualitative and quantitative study of the soil organic matter the determination of total carbon remains the most reliable, provided the nature of this organic matter is understood.

DETERMINATING DEFINITE FRACTIONS OF SOIL ORGANIC MATTER

In addition to determining total organic matter in the soil, numerous attempts have been made to analyze for certain definite organic fractions that were believed to play specific functions in the physical, chemical, or biological soil processes. The analysis of these fractions was based upon the old, totally unjustified assumption of Hilgard and others that plant residues added to the soil must be "humified" before the nutrient elements can become available for plant utilization. In other words, the "humified" organic matter was considered to be the intermediary material between the plant remains that have been introduced into the soil in the form of roots, stubble, green manures, and stable manures, and the final products of decomposition, such as ammonia and carbon dioxide. The complete lack of justification for this assumption has been amply illustrated in a previous contribution (18) in which the literature on the origin of the so-called soil "humus" has been reviewed in detail. The unwarranted conclusions seemed to have found support in the fact that in the decomposition of plant and animal residues in the soil various dark colored substances, possessing definite physical and chemical properties are produced. However, these dark colored substances can hardly be considered as intermediary decomposition products; they should rather be looked upon as substances resulting from direct or indirect decomposition of plant residues that have resisted further degradation as well as of products of microbial synthesis.

It is interesting to note, in this connection, that the soil chemists and agronomists have considered this "humified" organic matter largely from the point of view of its nitrogen content. The chemists, in their study of the nature of soil "humus," frequently left the nitrogen out of consideration altogether,

since the formulas suggested for the various "humic acids" were entirely free from nitrogen, although no treatment with strong alkalies or acids could remove this nitrogen completely without destroying the whole complex.

The various methods which have been suggested at different times for determining this "humified" portion of soil organic matter can be conveniently divided into two groups:

1. Methods based upon the use of alkalies for the extraction of the soil "humus." This "humus" or "humic acid" is either precipitated from the alkali solution by an acid, or, when ammonia has been used for extraction, the solution is evaporated leaving the "humus" residue. In all these determinations—including that of the *mulière noire* of Grandeau, "humic" and "hymetomelanic" acid of Hoppe-Seyler and Olen, and the α -fraction of the senior author—only a part of the soil organic matter, rarely more than 30 to 50 per cent, is usually obtained. The same thing is true of determinations based upon the depth of color of the alkali extract when compared with the color of an alkaline solution of a standard "humic acid" preparation, or some definitely colored solution. Because the chemical identity of this fraction is still a matter of discussion and because varying quantitative results were obtained depending on the nature and concentration of the alkali, on the temperature and length of extraction, and on the nature of precipitating agents (17, 19), this method may be abandoned for any accurate measurement of soil organic matter or of any definite fraction of it.

2. The oxidation of the "humified" portion of the soil organic matter by mild oxidizing agents, such as dilute hydrogen peroxide, permanganate solution, and hypochlorite solution. The value of all of these methods has been questioned, especially in view of the fact that we know nothing of the nature of the organic complexes which are acted upon by these reagents, as is shown later.

These two methods are based upon two distinctly different properties of a certain part of the organic matter, namely, solubility in alkalies and oxidation by certain weak oxidizing agents. Nothing definite is known concerning the chemical nature of these complexes; it is not known whether the two groups of methods deal with the same or with different chemical substances.

Springer, who reviewed these methods in detail recently (17), made an exception for the acetyl bromide method of Karrer. This reagent was found to dissolve natural plant constituents, but it leaves the so-called "humus" or newly formed, dark-colored substances intact. The method may yield interesting information in the study of decomposition of plant materials in the compost, such as in the differentiation between certain residual and resistant plant constituents and modified or synthesized complexes, but we can hardly expect that it may serve as a general method for determining the nature and abundance of soil organic matter.

For the purpose of throwing a certain amount of light upon the oxidation methods as a means of determining the nature of the particular organic complexes which are thereby oxidized, and of determining whether the substances thereby oxidized have any definite relation to the so-called "humus" complexes in the soil, the following experiments were carried out: Four preparations; namely, finely ground fresh chestnut wood, wood rotted by brown rot fungi and resulting in a product which consisted of 71 per cent lignin and modified

lignin complexes, forest soil consisting of surface material (F-layer) of a spruce forest, and lowmoor (sawgrass) peat from the Everglades, Florida. A detailed chemical analysis of the four materials is given in table 1.

Portions of the air-dried and ground materials were treated with a 6 per cent hydrogen peroxide solution, according to Robinson's method (8), and with a solution of chlorine dioxide (ClO_2), according to the method of Schmidt (11). The last method is known to dissolve all lignin in plant material and has found considerable application in plant chemistry.

TABLE 1
Chemical composition of four organic substances used for oxidation studies
In per cent of total dry material

CHEMICAL COMPLEXES	SOUND CHESTNUT WOOD	ROTTED WOOD	FOREST SOIL (F-LAYER)	LOWMOOR PEAT
Ether-soluble.....	2 66	1 48	3 58	2 98
Cold- and hot-water-soluble.....	7 08	1 26	5 14	1 73
Alcohol-soluble.....	3 27	5 05	1 06
Hemicelluloses.....	15 23	4 72	17 50	6 41
Cellulose.....	23 58	2 16	9 62	0 28
Lignins.....	22 05	71 14	42 26	46 12
Protein.....	0 54	1 31	6 84	23 06
Ash.....	0 54	0 65	6 05	10 00

TABLE 2
Influence of oxidation of fresh and decomposed plant residues with a six per cent solution of hydrogen peroxide and with a solution of chlorine dioxide

NATURE OF MATERIAL	PROPORTION OXIDIZED BY	
	H_2O_2	ClO_2
	per cent	per cent
Sound chestnut wood.....	20 1	28 0
Rotted wood.....	61 4	92 5
Forest soil.....	67 6	45 2
Lowmoor peat soil.....	80 2	73 0

Table 2 shows that a 6 per cent solution of hydrogen peroxide is capable of oxidizing not only decomposed or "humified" organic material but also a certain amount of fresh plant material; actually 20 per cent of the total sound chestnut wood was oxidized by this reagent. If that is the case, how much emphasis can be laid upon the determination of the so-called "humified" matter even in decomposed material by the use of this method? The rotted wood which contains 75 per cent lignin, as shown both by direct lignin determinations and by treatment with chlorine dioxide, is oxidized by hydrogen peroxide to the extent of 61 per cent. The lowmoor peat, which contains 10 per cent ash, 23 per cent nitrogenous compounds, various ether- and alcohol-soluble substances, hemicelluloses, and but 46 per cent of lignins and modified

lignin ("humus") complexes, was oxidized to the extent of 80 per cent by the peroxide reagent. One would hesitate to apply the term "humified" material to these 80 per cent of peat constituents, since a large part of it is no doubt of plant origin and since the term itself is meaningless in any attempt to interpret the chemical nature of the constituents as well as the processes of peat formation.

The chlorine dioxide reagent also reacts with various plant constituents, oxidizing more than the lignin fraction in the organic material. This is especially well brought out in the data on the sound chestnut wood and rotted wood. Here as well, it is rather difficult to interpret the results obtained by this method in terms of definite constituents of the decomposed plant residues. One would hardly be justified in any attempt to apply these results to an interpretation of the nature of the organic matter in soils and peats.

For the purpose of interpreting further the nature of those chemical constituents of the plant residues and of the decomposed organic matter which are acted upon by these oxidizing agents, definite quantities of the aforementioned four organic substances were treated with ether, then with hot alcohol, and finally with hot water. Various fractions of the four preparations thus extracted were now divided into several series and treated as follows: (a) One set was oxidized directly with hydrogen peroxide and chlorine dioxide reagents and the amount of material thereby lost determined. (b) Another set was boiled with 2 per cent hydrochloric acid, at 100°C. for 5 hours, to remove all the hemicelluloses. The residues were filtered, washed thoroughly with distilled water, and treated with the two oxidizing agents. (c) A third set of samples was treated with hydrochloric acid as in set (b), filtered, washed, dried, and then further treated with 10 volumes of 80 per cent sulfuric acid for 2 hours; this was then diluted with 15 volumes of water and boiled for 5 hours, the process resulting in a complete removal of the celluloses. The material left after this treatment consists of lignin with a small admixture of protein and ash; it was again subjected to the action of the two oxidizing agents. The data obtained as a result of the oxidation of these various preparations were calculated back to the original material and reported on a percentage basis (table 3).

From the results on the oxidation of the chestnut wood preparations by chlorine dioxide, one may conclude that this reagent acts primarily upon the lignin and to some extent upon the hemicelluloses of the plant tissues. With an increase in the lignin content of the preparation there is an increase in the amount of material removed by the chlorine dioxide reagent; finally when pure lignin is left, as a result of the acid treatment, 99.4 per cent of the material is oxidized by chlorine dioxide. However, the 6 per cent hydrogen peroxide solution acts only to a limited extent upon several of the plant constituents, including some of the lignin and some of the water- and alcohol-soluble constituents.

The rotted wood contained 71 per cent lignin, and one would, therefore, expect that it should be acted upon readily by the chlorine dioxide reagent; this

organic matter in the soil; however, to be able to study the various stages of decomposition of soil organic matter we must first gain a proper understanding of the mechanism of the decomposition processes; these are still imperfectly understood. 3. A study of the chemical composition of the soil organic matter; this has already been attempted by numerous investigators, and any new method of approach can only add further information to this complicated problem.

An attempt has already been made (21, 24) to apply the proximate system of analysis, which has been developed for the study of the processes of decomposition of plant materials, to the analysis of peat and forest soils, which are largely organic in nature. By this system of analysis, as much as 80 to 95 per cent of the constituents of these two organic soil formations have been accounted for in terms of definite chemical complexes.

One may now go a step further and apply this system of analysis to the study of the organic matter in inorganic soils. The method, of course, would have to be considerably modified, since one is dealing here with a mass of material containing only 1 to 10 per cent of organic matter and 90 to 99 per cent of inorganic constituents.

Keppeler (3) suggested that peat be treated with concentrated sulfuric acid solution (72 per cent), then the amount of reducing sugar produced be determined; the latter was found to serve as a good index of the degree of decomposition of peat: the less reducing sugar formed the more the peat is decomposed. Unfortunately, the fact was not considered that lowmoor, highmoor, and sedimentary peats are derived from different plant residues varying markedly in composition: although each can be compared with other peats of the same nature, they cannot be compared with one another, as has been shown elsewhere (22).

The carbon, nitrogen, and moisture contents of the untreated soil are determined on separate portions. The complete analysis can be carried out as follows: Two 100-gm. portions of air-dry soil are extracted first with ether in Soxhlets, for 12 to 24 hours. The ether extract is evaporated to a small volume, transferred to weighing bottles, dried to constant weight, and the amount of ether-soluble material determined. The ether-treated soil is next extracted with hot 95 per cent alcohol for 1 to 2 hours, on a water bath. The alcohol-soluble material is determined in the extract. The residual soil may then be treated with hot water; this is followed by extraction with 2 per cent hydrochloric acid solution, for 5 hours at 100°C.; the hot-water extract may be omitted and the soil treated with the acid following the alcohol extraction. The acid extract is filtered off and the soil washed with distilled water. The filtrate and washings are combined and made up to volume. One aliquot portion is used for a determination of reducing sugar, one for total nitrogen, and one for ammonia-nitrogen determinations. The total amount of organic matter that has gone into solution may be calculated from the carbon content in an aliquot portion of the extract. The sugar and the ammonia can serve as

indexes of the hemicellulose and amide content of the organic matter in the particular soil.

It should be noted here that after the soil has been treated with hydrochloric or with sulfuric acid, a solution is obtained which is frequently very rich in iron and aluminum. These elements interfere with an accurate sugar determination. After neutralization, the iron and aluminum precipitate should be filtered off, and the residue washed; the sugar is then determined in the filtrate. One must make sure that the results check well.

Aliquot portions (20 to 50 gm.) of the dry residue left after the hydrochloric acid treatment are now placed in flasks and 20 to 30 cc. of 80 per cent sulfuric acid solution is added. The acid is allowed to act upon the soil for $2\frac{1}{2}$ hours in the cold. The material is then diluted with 15 volumes of water (300 to 450 cc.) and boiled for 2 to 5 hours. This treatment results in the complete hydrolysis of the cellulose found in the soil and its transformation into glucose. The determination of the reducing sugar can serve as a fair index of the cellulose content of the soil. The residue from the sulfuric acid treatment is thoroughly washed with water, dried, weighed, and analyzed for total carbon and nitrogen. This residual organic matter consists of lignin and its transformation products and of various synthesized microbial complexes including certain organic nitrogen compounds. If one assumes that the distribution of the nitrogen in the organic nitrogenous soil complexes is the same as the nitrogen distribution in native plant and animal proteins, one can calculate the amount of organic nitrogenous substance and the residual material by multiplying the nitrogen content by the factor 6.25.² By subtracting from the total carbon found in the residue the carbon of the nitrogenous complex, assuming that the latter contains 50 per cent carbon and allowing 62 per cent carbon in the "lignin-humus" complex, one can calculate the amount of the "lignin-humus" complex in the residual soil and in the original soil. The following formula may be used for this purpose:

$$\text{Per cent of "lignin-humus" complex in soil} = \frac{a \times 100}{A} - \frac{b \times 100}{S},$$

where a = the carbon content in the sulfuric acid residue, calculated on the basis of the total original sample of soil,

A = the total carbon content of the sample of soil,

b = protein content in the sulfuric acid residue, obtained by multiplying the nitrogen content of the residue by 6.25; this is then calculated for the whole sample,

S = total organic matter in the soil sample, as calculated from the organic carbon of the soil.

² The use of the factor 6.25 for calculating protein or organic nitrogenous compounds from the total nitrogen of the soil may prove to be far from accurate. Before any more definite information has been obtained concerning the chemical nature of the nitrogenous compounds of the soil, the factor should be looked upon as tentative. It should be emphasized here as well that the factor 1.72 for determining the total organic matter, from the carbon content should also be considered as merely tentative.

It may prove desirable to subdivide this "lignin-humus" complex further into certain subgroups; this can be accomplished by the use of a reagent like acetyl-bromide, by the solubility of a part of the complex in ammonium hydroxide solution, or by the formation of a precipitate as a result of the acidification of the hot sodium hydroxide extract of the material.

For carrying out this analysis, several soils from different parts of the United States and Canada have been used. These soils can be described as follows:

- Soil no. 4. Summit soil from Missouri, Hor. A.
- Soil no. 6. Chernozem soil from Hays, Kansas, Hor. A.
- Soil no. 10. Alpine humus, from Pike's Peak, at a height of 13,800 feet.
- Soil no. 16. Chernozem soil from Edmonton, Alberta, Canada, taken at a depth of 1 to 25 cm.
- Soil no. 18. Brown soil at Indian Head, Saskatchewan, Canada, depth 1 to 20 cm.
- Soil no. 21. Chernozem soil from Brandon, Manitoba, Canada, depth 1 to 20 cm.
- Soil no. 22. Same as no. 21, 25 to 50 cm. depth.
- Soil no. 29. Carrington loam, dark colored, prairie soil, Hor. A.

A summary of the carbon, nitrogen, and organic matter content of these soils is given in table 4. These results show, first, that there is a considerable discrepancy in the organic matter content of the soil as determined by loss on ignition and as calculated from the carbon content. The loss on ignition is usually 35 to 75 per cent higher than the organic matter calculated from the carbon content and, in some cases even 118 per cent higher. Secondly, the fairly uniform ratio between the carbon and nitrogen of the soil is of considerable interest. This ratio ranges within narrow limits between 9.9 to 12.0.

Tabl. 5 gives the results of the proximate chemical composition of the organic matter found in these soils. These results were calculated on the basis of the total soil organic matter, the latter being determined from the total carbon figures given in table 4.

These results are highly interesting. They prove definitely that the chemical composition of organic matter in different soils varies considerably. This is true especially of the non-nitrogenous constituents. The fat and wax content (ether-soluble portion) varies from 0.46 to 4.71 per cent of the total organic matter. A similar variation is found in the amount of alcohol-soluble constituents (resinous substances). The celluloses and hemicelluloses in the soil also vary, although not to such an extent. It is interesting to note that the hemicellulose content of the soil organic matter is considerably larger than the cellulose content, just the reverse of what is found in plant residues. This is because the hemicellulose (including the pentosans and methyl-pentosans) group comprises also substances which have been largely synthesized by the soil microorganisms in the building up of their cell substance. It is interesting to note that some of the reducing sugars obtained in the cellulose fraction may represent not true plant cellulose but microbial cellulose or chitin.

The two largest groups of chemical complexes found in the soil organic

matter are the "lignin-humus" complex ("soil lignin" or "soil humus") and the nitrogenous compounds. These two groups make up 71 to 80 per cent of the total organic matter of the soil and an even larger amount of that part of the organic matter which is accounted for in these analyses. The high carbon content of the soil organic matter (usually taken as 58 per cent) is due to the predominance of the lignins, lignin-like complexes, and lignin derivatives with a high carbon content (62 to 64 per cent). The more or less definite relation

TABLE 4
The nature of the organic matter in different mineral soils

On the basis of dry soil

SOIL NO.	ORGANIC MATTER BY IGNITION	ORGANIC MATTER C \times 1.72	TOTAL CARBON	TOTAL NITROGEN	C/N	pH OF SOIL
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>		
4	7.89	4.49	2.61	0.2417	10.8	6.82
6	5.98	2.74	1.59	0.1543	10.3	7.56
10	11.00	7.74	4.50	0.4429	10.1	5.08
16	17.10	11.20	6.51	0.6695	9.9	6.38
18	10.34	6.24	3.63	0.3324	10.9	8.26
21	10.06	7.40	4.30	0.3950	10.9	8.25
22	12.03	8.31	4.83	0.4403	11.0	8.26
29	10.16	6.48	3.77	0.3145	12.0	7.75

TABLE 5
Proximate chemical composition of soil organic matter
On the basis of the total soil organic matter (C \times 1.72)

SOIL NO.	ETHER- SOLUBLE MATERIAL	ALCOHOL- SOLUBLE MATERIAL	HEMICELLU- LOSES	CELLULOSE	LIGNIN- HUMUS COMPLEX	ORGANIC NITROGE- NOUS COMPLEXES	SUM OF THE CONSTITU- ENTS ACCOUNTED FOR
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
4	3.56	0.58	5.44	3.55	43.37	33.78	90.28
6	4.71	1.53	8.60	5.22	40.81	34.74	95.61
10	1.94	3.10	12.59	5.36	35.18	35.77	93.94
16	0.80	0.82	5.53	4.12	41.87	37.35	90.49
18	1.02	0.88	6.96	3.50	42.05	33.25	87.66
21	0.46	0.84	8.54	2.83	42.83	33.36	88.86
22	0.52	0.63	10.66	3.38	46.50	33.13	94.82
29	0.62	0.61	8.21	3.64	49.29	30.38	92.75

between the "lignin-humus" complex and the organic nitrogenous complexes accounts for the more or less constant ratio between the carbon and the nitrogen in the soil organic matter.

The close correlation between these two groups of complexes in the soil organic matter may be a matter of chance or it may be due to the actual formation of a chemical compound between the two, whereby they are made more resistant to the action of the microorganisms. It should be recalled

in this connection that the peats, especially the lowmoors, which are more closely related in their hemicellulose and cellulose content to the soil organic matter, are richer in lignins and lower in nitrogenous compounds than the organic matter of the soil; this explains the considerably wider carbon-nitrogen ratio of the peat material than of the soil organic matter.

Special attention should be called to soil 10, or the Alpine soil. Because of the very high altitude and prevailing low temperature, the organic matter produced by the roots and by other residues of the Alpine vegetation is not decomposed as readily as in normal field soils with a more optimum temperature for the activities of soil microorganisms. The high cellulose and hemicellulose content, the high ether- and alcohol-soluble fractions, as well as the low "lignin-humus" content point to the fact that this soil still contains some plant residues undecomposed or in the process of active decomposition.

These results bring out definitely the fact that by the method of analysis proposed here one may be able to account for 90 per cent or more of the soil organic matter in the form of definite chemical compounds. Although the exact chemical identity of some of the compounds may be questioned, still there is no room here for the application of such indefinite terms as "humus," "humified," "humic matter," as well as of the numerous "humic acids," which have been confusing, both as to their nature and their origin, to the investigator who has attempted to contribute to our knowledge of the soil organic matter. This method of proximate analysis can certainly not be recommended for a routine study of the nature of soil organic matter but it enables us to investigate the exact chemical identity of the constituents of the organic matter of the soil, just as a proximate analysis of plant material allows us to make a closer study of the nature of the chemical complexes in the plant which are still unknown.

The advantage of dividing the soil organic matter into several groups by the method proposed here over the alkali methods previously employed can hardly be questioned. Whatever the nature of the fatty, waxy, and resinous substances present (15) in various soils under different conditions of treatment, their grouping together into the ether- and alcohol-soluble fractions allows us to think of them in definite chemical terms. Whatever the nature of the hemicelluloses, whether they are pentosans and methyl pentosans, the presence of which has been demonstrated in soil by Michelet and Sebelien [about 4.2 per cent of the organic matter (6)], by König and associates (4), by Schreiner and Shorey (14), and by others, or hexosans such as galactans and mannans, or whether the sugar is obtained by hydrolysis of nucleo-proteids, oxycelluloses, pectins, and various uronic acid compounds, their classification into one group places them conveniently for further investigation. The same thing is true of the celluloses. Soil no. 16, for example, which is reported to contain 5.53 per cent hemicellulose and 4.12 per cent cellulose (on the basis of the soil organic matter), gave 6.85 per cent furfural-yielding substances, on distillation with 12 per cent hydrochloric acid. The two remaining groups, namely,

the organic nitrogenous compounds, which can be referred to as the "protein group," and the lignins or lignin-like complexes and modified lignins, which can be referred to as the "lignin" or the "lignin-humus" group, comprise 70 to 80 per cent of the total soil organic matter. The "protein" group, on hydrolysis with acids and alkalis, gives various amino acids, acid amides, and heterocyclic compounds. The relative amide content is considerably greater than in plant or animal proteins, but the nature of most of the heterocyclic compounds as well as of the melanoid-like compounds (12), still remains to be determined. The presence of the lignins and lignin-like complexes in soil can also be demonstrated by methoxyl determinations (6, 4, 16).

In all the past "humus" and "humic acid" determinations, when the soil was treated with an alkali solution at a high or low temperature for a short or a long period of time, several of the chemical complexes discussed here were acted upon; namely, the hemicelluloses, the "lignin" complex, and the "protein" groups; they were partly brought into solution and partly hydrolyzed depending upon the extracting agent and upon conditions of extraction. This action is very similar to that which results from the treatment of undecomposed plant material with an alkali solution, with the sole exception that, in the case of the soil, the problem is further complicated by the presence of a large amount of aluminosilicates which are also brought into solution by the alkali. When an acid is added to the alkaline soil extract a precipitate is formed, the amount and nature of which (such as nitrogen and ash content) depend upon the amount and nature of the precipitating agent and the temperature of precipitation. An exact similarity is found in the precipitation of the alkaline plant extract by acids. It is this precipitate which was termed "humic acid" and was frequently further separated on the basis of solubility in alcohol, in pyridine, and in other solvents. One can easily show that this "humic acid" is nothing but a mixture of: (a) certain hemicelluloses (in the broadest sense), since it gives reducing sugars on hydrolysis with dilute acids and furfural on distillation with 12 per cent hydrochloric acid; (b) nitrogenous organic compounds since it usually contains 3 per cent nitrogen—part of which is readily hydrolyzed with dilute acids, giving amino acids and acid amides, and part of which cannot be hydrolyzed even by prolonged boiling with 20 per cent hydrochloric acid, pointing to its non-protein nature; (c) lignins and lignin-like complexes, as shown by its resistance to the action of 80 per cent sulfuric acid in the cold and by its methoxyl content. These phenomena will be discussed in detail in a later contribution.

One of the most important and interesting groups of chemical complexes in the proximate analysis of plant material, composted material, and soil organic matter is found in the "protein" group or the organic nitrogenous compounds. It should be recalled here that the percentage of "protein" in the soil organic matter is calculated by multiplying the total nitrogen of the soil (the water-soluble forms of nitrogen in ordinary field soil being quite negligible) by 6.25; the quantity thus obtained is divided by the total organic matter of the soil (carbon content $\times 1.72$) and multiplied by 100.

One assumes thereby that the nitrogen content of the soil nitrogenous organic complexes is 16 per cent, which is probably far from accurate, and that the carbon content of the soil organic matter is 58 per cent, which is also somewhat too high, especially in view of the carbohydrate content of the organic matter. These figures must be reinterpreted with the further accumulation of our knowledge of the chemical composition of the soil organic matter.

Given a constant carbon-nitrogen ratio in the soil organic matter and constant factors for nitrogen and carbon, the percentage of "protein" in soil will be expected to be constant, as is actually the case. Should the C/N ratio always be 10, the theoretical amount of "protein" or hypothetical nitrogenous compounds in soil would be:

$$\frac{N}{C} \times \frac{\text{protein factor}}{\text{total organic matter factor}} \times 100 \text{ or } \frac{1}{10} \times \frac{6.25}{1.72} \times 100 = 36.34 \text{ per cent}$$

The "protein" content in soils 10 and 16 (table 5), which have a carbon-nitrogen ratio approaching the theoretical 10:1, more closely than that of the other soils, is actually found to be very close to this theoretical figure, namely 35.77 and 37.35 per cent. The wider the C/N ratio, the lower is the "protein" content of the soil organic matter; the narrower the ratio, the higher is the "protein" content.

The question then arises: What is the nature of these nitrogenous compounds? There is no doubt that they are partly protein-like in nature, because on hydrolysis with acids they give a large amount of various amino acids. However, the distribution of the amino groups in the proteins of the soil organic matter is quite different from that in the plant or animal proteins. As a matter of fact, Lathrop (5) and Morrow (7) have shown that no matter what the amino acid distribution in the proteins added to the soil, the newly formed soil "protein" is quite different in nature, and that different soils behave alike in producing the same type of protein. This points definitely to the fact expressed by the senior author repeatedly concerning the rôle of the microbial cell substance as a source of protein or of organic nitrogenous complexes in the soil organic matter.

The following experiment deals, in a preliminary manner, with the distribution of nitrogen in the various soils previously analyzed by repeated treatment with different concentrations of acid (table 6). Only between 22.5 and 33.5 per cent of the total soil nitrogen is made soluble by prolonged boiling with 2 per cent hydrochloric acid. The amount is increased to 38.4 per cent in the Alpine soil, which, as the general analysis shows, is still rich in undecomposed organic matter. Taking into consideration the average of all these soils, we find that 29.5 per cent of the nitrogen in the soil organic matter is made soluble in dilute acid, of which 7.6 per cent, or a little more than a quarter, of the nitrogen thus made soluble, is in the form of ammonia. This form of nitrogen is derived from the amides, which become hydrolyzed by the acid treatment. An almost equal amount (average 29.6 per cent) was made further soluble by

treatment with cold 80 per cent sulfuric acid followed by boiling with a 5 per cent solution of this acid for 5 hours.

When the residual soil after the two acid treatments was boiled with a 20 per cent hydrochloric acid solution for 30 hours, another 18 per cent of the nitrogen was made soluble; the residual soil still contained, on an average, 16.3 per cent of the original nitrogen. Whether this form of nitrogen, which is so highly resistant to the action of concentrated acid, is also most resistant to the action of microorganisms still remains to be determined. It is known at least that this form of nitrogen is low in plant proteins; the lowest amount of this nitrogen was found in the Alpine soil, considerable amounts of the organic matter of which are still in the process of decomposition.

TABLE 6

Influence of acid concentration and time of action upon the solubility of the "protein" nitrogen of the soil

SOIL NO.	TOTAL NITROGEN IN 100 GM. OF DRY SOIL	NITROGEN MADE SOLUBLE BY BOILING WITH 2 PER CENT HCl FOR 5 HOURS				NITROGEN MADE SOLUBLE BY 80 PER CENT SULFURIC ACID IN COLD (2 HOURS) FOLLOWED BY BOILING IN 5 PER CENT H ₂ SO ₄ FOR 5 HOURS		NITROGEN MADE SOLUBLE BY BOILING WITH 20 PER CENT HCl FOR 30 HOURS		"HUMIN" NITROGEN LEFT IN RESIDUE AFTER BOILING WITH 20 PER CENT HCl FOR 30 HOURS		TOTAL NITROGEN ACCOUNTED FOR
		Nitrogen in solution		Ammonia in solution		mgm.	per cent of total	mgm.	per cent of total	mgm.	per cent of total	
		mgm.	per cent of total	mgm.	per cent of total N							
4	239	64.8	27.1	23.1	9.7	68.7	28.7	53.4	22.3	37.2	15.6	93.7
6	154	51.6	33.5	12.7	8.2	52.7	34.2	33.5	21.8	18.1	11.8	101.3
10	443	170.1	38.4	43.8	9.9	133.8	30.2	48.1	10.9	41.2	9.3	88.8
16	670	151.1	22.5	41.4	6.2	202.4	30.2	104.0	15.5	176.5	26.3	94.5
18	332	85.8	25.8	24.4	7.3	104.4	31.4	82.3	24.8	56.2	16.9	98.9
21	398	117.4	29.5	30.4	7.6	92.8	23.3	62.4	15.7	82.8	20.8	89.3
22	440	121.6	27.6	33.5	7.6	116.1	26.4	70.7	16.1	84.5	19.2	89.3
29	315	100.0	31.7	26.9	8.5	102.0	32.4	51.9	16.5	33.4	10.6	91.2

In only one instance among the various soils analyzed, was there more than one horizon used, namely, in the case of the Brandon soil, with A and B horizons (nos. 21 and 22). The lower horizon was richer in organic matter than the upper horizon, as is shown by the higher carbon and nitrogen content, but the nature of the organic matter was nearly identical in both instances. This can be readily seen from the almost exact carbon-nitrogen ratio, and from the nitrogen distribution, as well as from the relative abundance of the non-nitrogenous complexes. The reaction of both horizons was also nearly the same. These results, as well, point to the possible applications of the method to the study of the nature of soil organic matter.

The analyses of two other horizons of one soil taken from different profiles of a solonietz type of soil near Fargo, North Dakota, are recorded in table 7. In the analyses of these two soils, the cold- and hot-water-soluble portions of the organic matter were also included. The results agree fully with the proximate analyses of the other soils and show again only minor differences in the chemical

composition of the two horizons, which, however, may prove important when more information has accumulated.

Two theories are current at present in the literature in regard to the chemical nature of the soil organic matter: According to one theory, the soil "humus" is made up of a few simple, well-defined chemical compounds, the so-called "humic acids" and "humins," which are believed to be produced from plant and animal residues added to the soil by certain simple chemical or biological processes. According to the other theory (14), the soil organic matter is made up of a large number of chemical constituents. Numerous organic compounds can be isolated from the soil, provided the proper methods are available.

The proponents of the first theory sought to clarify our conception of the nature of soil organic matter by determining the chemical and physico-chemical properties of two or three of the "amino acids." In most cases, however, these

TABLE 7

The chemical nature of the organic matter of two horizons of a Chernozem profile, at Fargo, N. D.

ORGANIC COMPLEXES	HORIZON A	HORIZON B
Organic matter by ignition..... per cent	12.63	12.95
Total carbon..... per cent	4.80	5.09
C \times 1.72.....	8.26	8.75
Total nitrogen..... per cent	0.400	0.379
C/N.....	12.0	13.0
Proximate chemical composition, on per cent basis of total organic matter (C \times 1.72)		
Ether-soluble.....	2.72	2.18
Cold-water-soluble.....	1.16	1.01
Hot-water-soluble.....	1.31	1.24
Alcohol-soluble.....	0.98	0.91
Hemicelluloses.....	1.81	2.75
Cellulose.....	6.12	4.86
Lignin-humus complex.....	47.70	45.82
"Proteins" or organic nitrogenous compounds.....	30.27	27.08

acids were nothing but labels for compounds obtained by different methods of preparation rather than of well-defined chemical properties. The proponents of the second theory did not attempt to present a clear picture of the soil organic matter as a whole, if that is at all possible.

The results presented here demonstrate beyond any doubt that a detailed study of the chemical nature of soil organic matter will reveal the fact that it is very complex in composition. The complexity in chemical composition of plant materials and animal bodies has not prevented the plant chemist and animal biochemist from undertaking a detailed study of them, with the result that a great body of information on these two subjects has accumulated; the complexity of the soil organic matter as well need not prevent the chemist, preferably the organic chemist, interested in soil problems from investigating the nature of this organic matter.

In the study of soil chemistry, the greatest emphasis has been laid upon the inorganic constituents while the organic chemical problems have received but scant attention. Our knowledge of the organic chemistry of the soil, especially of that highly important group of soil constituents, the organic matter complex in nature and formed as a result of numerous microbiological processes of decomposition and of synthesis, is still a science to be developed. For the present, however, a simplified method based upon the more detailed analysis may be suggested here, which will enable the student of soils to obtain at least a certain insight into the nature of the organic matter in the various soils and serve as a beginning for further investigation.

SIMPLIFIED METHOD OF ANALYSIS OF SOIL ORGANIC MATTER

The methods previously utilized for the study of the chemical nature of soil organic matter are somewhat too complicated for routine analysis. An attempt has been made, however, to simplify the method of analysis. The following is a brief outline of such a simplified method:

The soil to be analyzed is air-dried, ground, and sieved. The air-dry material may be used for analysis but the results should be calculated on an oven-dry basis. Total nitrogen and total carbon determinations are made on the air-dry material. The results of the analysis of the organic matter should be calculated on the per cent basis of the total organic matter of the soil. This is obtained by multiplying the organic carbon content of the soil by 1.72. This factor will probably be modified when considerable information has accumulated concerning the chemical composition of the soil organic matter. For the present, however, this factor is as satisfactory as any other one (.20 for example) that might be suggested.

Two 200-gm. quantities of the oven-dry soil are extracted with ether in Soxhlets for 10 to 16 hours. This is followed by hot alcohol extraction. The extraction with a mixture (1:1) of benzol-alcohol may be employed, following the ether extraction, or may be used in place of both ether and alcohol extractions. The ether extract is evaporated to a small volume, then transferred to a weighing bottle; the residue carefully dried and the bottle weighed. The alcoholic extract is evaporated in weighed dishes. The amount of material (fats, waxes, and resins) soluble in ether and in alcohol is thus obtained. The extraction with benzol-alcohol mixture, in place of the ether and alcohol extractions, is carried out in a similar manner. The material extracted from soil by benzol-alcohol was found to be nearly equivalent to that extracted by ether and by alcohol.

Fifty-gram quantities of the dried residual material are placed in beakers and treated with 25 cc. of 80 per cent sulfuric acid solution for 2½ hours in the cold. Next, 375-cc. portions of distilled water are added to the beakers and the diluted acid extract is heated for 5 hours in flowing steam. The digest is filtered through dried and weighed papers and the residue is washed thoroughly with water. The solution is made up to volume and the amount of reducing sugar (also total and ammonia nitrogen if desired) determined. The sugar serves as a measure of the total carbohydrate content of the soil organic matter.

The residue from the acid extraction is dried and weighed. Two 5-gm. quantities from each residue are used for total carbon determinations, and two 10-gm. quantities are used for total nitrogen determinations. The "lignin" or "lignin-humus" complex is then calculated from the carbon and nitrogen content of this residue.

A lowmoor peat and two Sassafras soils, one manured for a number of years and one unmanured, were used for this analysis. The results are given in

table 8. These results show that by this method of analysis one can account for 95 per cent of the soil organic matter in the form of four definite, more or less well-defined chemical groups.

This method has a number of distinct advantages over the alkali extraction methods frequently used formerly:

It enables us to determine at a glance the stage of decomposition of the organic matter in the soil. Fresh, slightly decomposed organic matter of plant origin will have a high carbohydrate fraction and low "protein" and "lignin-humus" fractions. The more advanced the stage of decomposition of the organic matter, the less will be the carbohydrate content and the larger will become the last two fractions.

The method enables us to divide the organic matter of the soil into groups that are convenient for further study. Each fraction can then be studied by itself.

The results brought out by this method show how fallacious it is to speak of "humified" and "non-humified" portions of the soil organic matter. One may speak, however, of the

TABLE 8
Proximate composition of soil organic matter
On the basis of organic matter content of soil*

NATURE OF SOIL	ETHER- AND ALCOHOL SOLUBLE FRACTION	CARBOHY- DRATE FRACTION, AS SUGAR	"PRO- TEIN"	"LIGNIN- HUMUS"	SUM OF ORGANIC MATTER AC- COUNTED FOR
	per cent	per cent	per cent	per cent	per cent
Lowmoor† peat 51.....	2 51	10 29	25.97	54.48	93.25
Manured soil,‡ 5A.....	2 75	12 78	30.83	43.36	89.72
Unmanured soil, 7A.....	2 88	10 58	34.57	42.32	90.35

* In the case of the mineral soils, the organic matter content is calculated by multiplying the organic carbon by the factor 1.724.

† The peat analysis is also calculated on an ash-free basis; the ash content of the peat was 10 per cent.

‡ Total carbon content of the manured soil is 1.75 per cent; of the unmanured, 0.82 per cent.

degree of decomposition as indicated by the chemical changes that have taken place in the distribution of the various constituents in plant residues after they have become incorporated in the soil and become a part of its organic matter.

SUMMARY

The most reliable method available at present for determining quantitatively the soil organic matter is based upon the determination of organic carbon, which is multiplied by 1.724 to give total soil organic matter.

The methods proposed at different times for measuring the abundance of "humus" or "humic acid" in the soil, based upon its extraction with alkalis, cannot be used for determining quantitatively the soil organic matter or even a definite fraction of it. In these methods the extraction of the soil with alkalis is either followed by the evaporation of the alkali, by the precipitation of the

so-called "humus" with an acid, or by the determination of the color of the solution extracted, which is then compared with a standard solution.

Those methods which are based upon the use of oxidizing agents, such as permanganate solution or 6 per cent hydrogen peroxide solution for partial oxidation of soil organic matter, cannot be used for determining its degree of decomposition. These reagents usually act upon different substances, partly of plant origin and partly synthesized by the microorganisms, which have no direct bearing upon the degree of decomposition of the plant organic matter.

A proximate method of analysis of soil organic matter is suggested, whereby 90 to 95 per cent of all the constituents of the organic matter of the soil can be accounted for in groups of definite chemical complexes.

It has been found, by this method of analysis, that the soil organic matter is made up largely of two groups of complexes: the lignin-like complexes, which can be referred to as "humus-lignin" or "soil-lignin;" and the nitrogenous complexes or "soil proteins." In addition to these two groups, the soil organic matter contains some fats, waxes, and resinous substances (ether- and alcohol-soluble substances), and certain carbohydrates, including various hemicelluloses and small amounts of cellulose-like substances.

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AVAILABILITY OF MANGANESE AND OF IRON AS AFFECTED BY APPLICATIONS OF CALCIUM AND MAGNESIUM CARBONATES TO THE SOIL¹

H. B. MANN²

North Carolina Agricultural Experiment Station

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One of the first great contributions to plant production was the establishment of the fact that certain elements are essential to plant growth, which culminated in Liebig's complete list, from which he erroneously omitted iron. With this change, the original 10 essential elements have remained intact for about three-quarters of a century, although many investigators have produced evidence favorable to the inclusion of others.

The importance of manganese has been stressed, but until recently it has received little consideration. Within the past few years the coincidence between response to manganese fertilization and the alkalinity of certain naturally calcareous and limed soils has indicated a probable relation between soil reaction and the availability of manganese similar to that which has been apparently established for iron.

HISTORICAL REVIEW

Manganese

The distribution of manganese in nature has been extensively studied. Richard (40), Bertrand and Rosenblatt (2), and McHargue (25) have determined its distribution and localization in plants, and Robinson (41) has recently reported the distribution of manganese in soils and shown that the amount varies throughout a wide range. High concentrations of manganese in the soil have been observed to be toxic to plant growth by Ewell (7), Lindsey (23), Kelly (21), and Guthrie and Cohen (15). On the other hand, Skinner and Reid (46), McHargue (26), McLean and Gilbert (29), Schreiner (44), Skinner (45, 38), and Willis (51) have suggested a deficiency of this element in certain soils.

Little is known of the rôle of manganese in plant nutrition but many theories have been advanced to account for its effect on plant growth. A stimulating

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effect of small quantities has been reported by Nagaoka (34), Labergerie (22), Bertrand (1), and Molinari and Ligot (33); whereas Grandeau (14) and Carlier and Claussen (4) obtained no appreciable benefit from its use. Manganese has been associated with the formation of nitrogenous compounds in plants by Salomone (43), Olaru (36), Roscosolano (42), and Pietruszczynski (37). Skinner et al. (47), (46), from early investigations, were led to conclude that crop response from manganese was related to its effect on the oxidative processes of the soil. Later work by Deatrich (6) seemed to substantiate this. That manganese acts both as a fertilizing agent and a catalytic body was suggested by Boucher (3). However, the general acceptance of manganese as necessary for plant economy began with the work of McHargue (24) and the evidence appears conclusive with the observation by McLean (28) that a manganese solution forced into the leaves of chlorotic plants through the stomata was locally as effective as was the application of the manganese to the soil.

The works of Somay (48), Masoni (30), Neotin (35), Ricci and Barbera (39), and McHargue (26) indicate that any kind of acid increases the solubility or availability of manganese, although only small amounts of the total manganese in soils are soluble in water.

Iron

The conditions governing the availability of iron have been extensively reported by Gile and Carrero (12), Jones and Shive (20), Mazé (31), and Carr and Brewer (5), but, as shown by the work of Willis and Carrero (52), the solubility of iron and the amount available to plants cannot be taken as a certain determinant of the efficiency of iron. The hypothesis advanced by Hopkins and Wann (17) that iron is active only in the ionized form offers a very good explanation for this.

Manganese and iron relations

Masoni (30) found the solubility of manganese and iron similar on both acid and limed soils. Johnson (18), on the other hand, offers good evidence that inorganic iron compounds may be held so completely oxidized in the soil in the presence of native manganese as to be practically insoluble. Fulsutome (8) obtained very little response from either iron or manganese when used singly, but obtained marked effect upon growth from joint applications.

A deficiency of any one of several elements will result in the failure of plants to develop a normal green color (32). Such an iron chlorosis has been reported by Gile (10, 11, 13) and by Johnson (18, 19); a chlorosis on heavily limed soils apparently associated with manganese deficiency has been observed by Hartwell (16), McLean and Gilbert (29), Gilbert, McLean, and Hardin (9), and Zimmerley (53).

The conflicting results obtained from the use of manganese and iron indicate that a response from these elements when applied to the soil can only be

expected under certain conditions, and that a chlorosis of plants may be due to a deficiency of either. In some of the tropical soils iron seems to be deficient, but in the Atlantic Coastal Plain the chlorotic condition of certain plants grown on the naturally calcareous soils and those receiving large applications of calcitic or dolomitic lime is apparently due to a manganese deficiency. This investigation was therefore undertaken to determine the availability of manganese and of iron as affected by applications of calcium and magnesium carbonates to the soil.

SOLUBILITY OF MANGANESE AND OF IRON IN DUNKIRK AND DUNBAR SOILS

Carr and Brewer (5) have shown that the solubility of manganese and iron in solution decreases as the H-ion concentration of the solution is decreased. It cannot be assumed, however, that the solubility in soils will be the same as in solutions. This experiment was made to determine the solubility of these elements in soils as affected by applications of calcium and magnesium carbonates.

Water-soluble iron and manganese in Dunkirk gravelly sandy loam

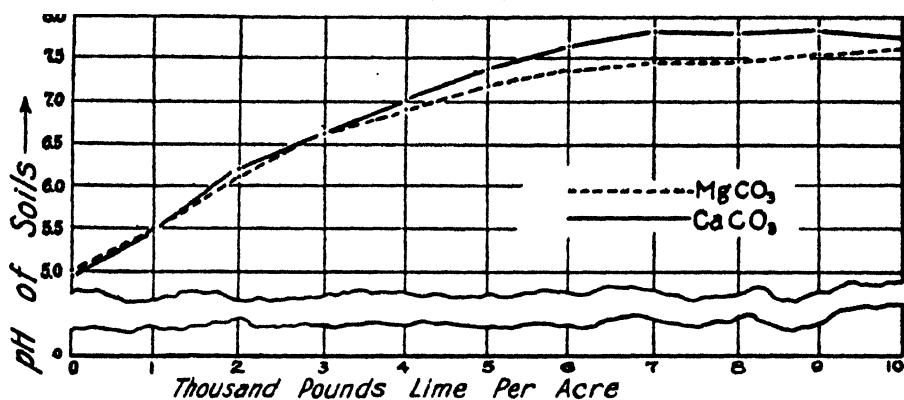
On February 23, 1928, air-dry Dunkirk gravelly sandy loam soil was pulverized and all rocks and roots were removed. Four kilograms of this soil was then thoroughly mixed and put into 1-gallon glazed pots. Duplicate pots were treated with equivalent amounts of calcium and magnesium carbonates varying in increments of 1,000 pounds from 0 to 10,000 pounds an acre.

The moisture content of this soil was maintained at 10 per cent of the air-dry weight with distilled water until June 9, when samples were taken representing each treatment, and H-ion concentration determinations were made by the quinhydrone method. A sample equivalent to 1,000 gm. of soil on an air-dry basis was also taken for the determination of water-soluble iron and manganese. Each of these samples was placed in an 8-liter glass bottle and agitated with 5,000 cc. of distilled water at regular intervals for 48 hours. The solution was then filtered through paper and 4,000 cc. of the filtrate evaporated to dryness over steam. The residue was twice taken up with concentrated nitric acid and evaporated to dryness. A 10-cc. aliquot of this soil extract concentrate was used for the determination of iron by the thiocyanate method as described in the Bureau of Soils Bulletin 31, 1926.

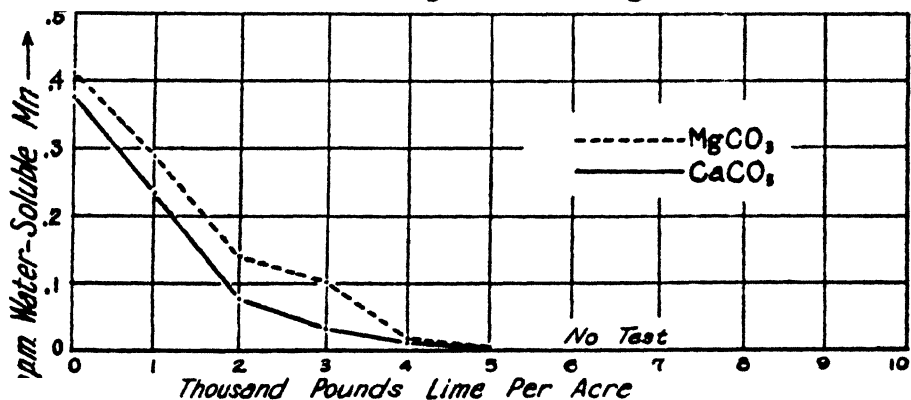
Twenty-five cubic centimeters of the soil extract concentrate was used for the determination of manganese by the periodate method of Willard and Greathouse (50) with minor modifications. Considerable difficulty was experienced in developing the permanganate color in the soil extract concentrates from soils receiving the heavier rates of application, especially of magnesium carbonate, on account of high concentrations of organic matter dissolved from the soils.

Increasing amounts of calcium and magnesium carbonates were attended by progressive decreases in the H-ion concentration although the two alkaline

On The H-ion Concentration



On The Solubility Of Manganese



On The Solubility Of Iron

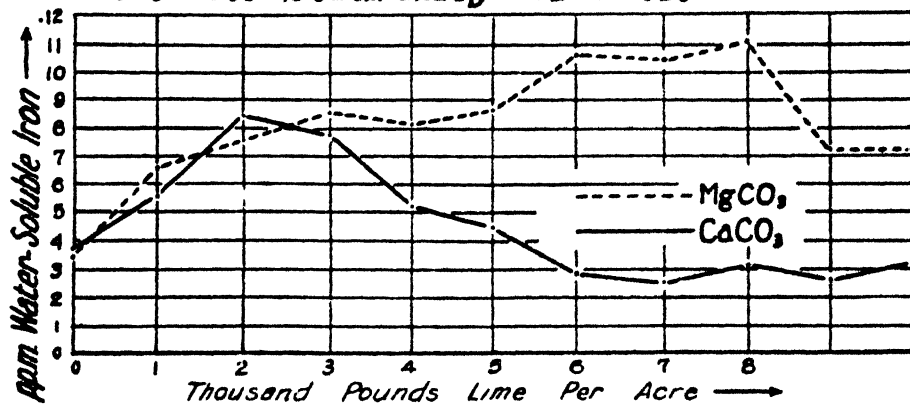


FIG. 1. EFFECT OF APPLICATIONS OF CALCIUM AND MAGNESIUM CARBONATES TO DUNKIRK GRAVELLY SANDY LOAM

agents were not equally effective in this respect, the differences being clearly shown by the H-ion concentration curves in figure 1. When the increments in pH are plotted as arithmetical rather than logarithmic values, the decrease in acidity with increase in calcium carbonate is approximately uniform, up to the 6,000-pound application, at a pH of 7.67 with minimum H-ion concentration of pH 7.8, reached with the 7,000-pound rate.

Increases in pH with increased magnesium carbonate were also uniform to about pH 7.19, which was reached with the rate equivalent to 5,000 pounds of calcium carbonate. Greater rates of application increased the pH up to the maximum at the 10,000-pound equivalent rate.

The effect of liming on the solubility of soil manganese was practically independent of the materials used. As shown by the manganese solubility curves in figure 1, there was a rapid and uniform decrease in solubility from the unlimed soil to the 2,000-pound rate of application, at a pH of about 6.2, with a gradual decrease to the 4,000-pound rate, at approximate neutrality. No manganese was found in extracts of the soil receiving more than 5,000 pounds of calcium carbonate or its equivalent in magnesium carbonate. Failure to show traces of manganese at the high rates was probably due to limitations of the analytical method.

In contrast to the regularity of the solubility curve for manganese is that for iron (figure 1). With the calcium carbonate treatments there is a sharp increase in solubility of iron up to a maximum concentration of 8.44 p.p.m. of soil at the 2,000-pound rate of application. Thereafter there is a gradual decrease in solubility to a virtual minimum at the 6,000-pound rate. With the magnesium carbonate treatments there is an increase in solubility to a maximum of 11.13 p.p.m., at the 8,000-pound equivalent rate, followed by a sharp decrease at the higher rates.

Obviously there is no simple relation between solubility of iron and reaction, or rate of liming, as was the case with manganese, nor is there a similarity between the effects of calcium and magnesium carbonates.

The shape of the solubility curve suggests the influence of two factors having opposite effects on the solubility of iron. One of these is probably the reaction tendency of the soil toward precipitation at the lower H-ion concentration, whereas the other may well be the increased solubility of iron in combination with the organic matter observed in the soil extracts at the higher rates of liming. The observation that greater amounts of organic matter appeared to be dissolved by the magnesium carbonate treatment would explain the divergence between the solubility curves with the calcium and the magnesium carbonates.

Water-soluble iron and manganese in Dunbar fine sandy loam

The soil used for this investigation was taken from an unfertilized and unlimed plat in a fertilizer experiment which had been conducted on the Pender County Branch Station Farm for 15 years. The soybeans grown in a

3-year rotation showed, on the limed end of the plat, a characteristic chlorotic condition of the upper leaves whereas those on the unlimed end were of normal green color.

On October 10, 1928, 4,000 gm. of this soil in air-dry condition was treated with equivalent amounts of calcium and magnesium carbonates varying from 0 to 10,000 pounds an acre and put into clean 1-gallon glazed pots. These potted soils were brought up to 10 per cent moisture with distilled water and maintained at this moisture content for approximately three months.

On January 15, 1929, samples were taken from each pot for the determination of water-soluble iron, manganese, and H-ion concentration. The H-ion concentration was determined by the quinhydrone electrode method, as in the case of the Dunkirk soil, and the soil extract for the determination of water-soluble iron and manganese obtained and concentrated as before.

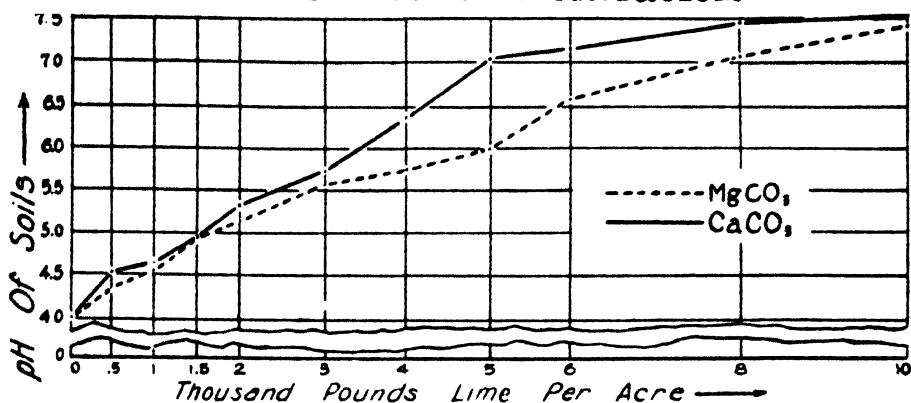
For the determination of iron the colorimetric method of Stokes and Cain (49) was used, as it gave more consistent results than the method used with the Dunkirk soil. Colors were compared in a Bosch and Lomb colorimeter with standards of nearly equal concentration. For the determination of water-soluble manganese it was found that digestion with a small amount of sulfuric acid and potassium chlorate destroyed all traces of organic matter, permitting a more satisfactory development of color in the extracts of the heavily limed soils. Otherwise the method of analysis was essentially the same as with the Dunkirk soil. Treatments and analytical data are presented graphically in figure 2.

As with the Dunkirk soil, increases in the rate of liming progressively decreased the H-ion concentration, and the rate of decrease was uniform for both calcium and magnesium carbonates up to the 1,500-pound rate. From this point the rate of increase, as shown by the H-ion concentration curves in figure 2, was less rapid with magnesium carbonate, and no maximum was reached with the latter material at the heaviest rate of application, giving a pH of 7.4.

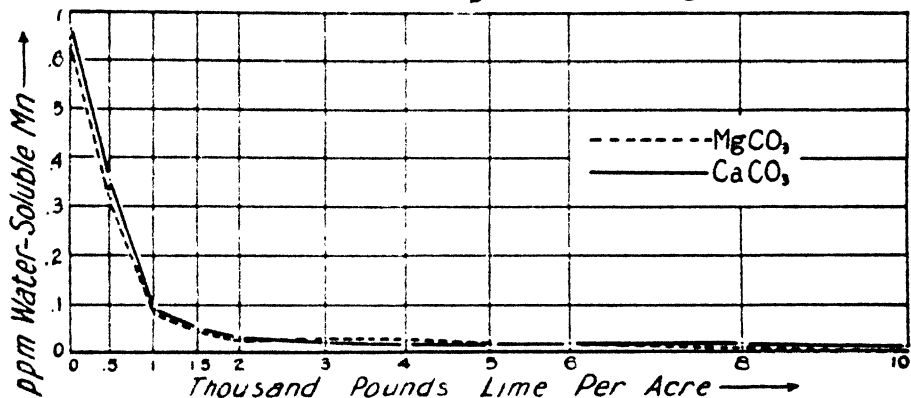
Increased rates of application of calcium carbonate uniformly increased the pH values up to the 5,000-pound rate and an approximate minimum H-ion concentration was reached at the 10,000-pound rate, with a pH of 7.53.

With allowances for the greater initial acidity of the Dunbar soil there is a striking similarity in the reaction curves of the two soils as regards both the calcium and magnesium carbonates. Calcium carbonate equivalent to 3,500 pounds an acre was required to raise the pH of the Dunbar soil from 5 to 7, whereas 4,000 pounds was required by the Dunkirk soil, indicating a slightly greater buffer value for the latter. Manganese was distinctly more soluble in the Dunbar than in the Dunkirk soil, both unlimed, as would be expected on account of the greater acidity of the Dunbar soil. The decrease in the solubility with increased rates of liming was much the sharper with the Dunbar soil. Probably on account of improved technique, determinable amounts of manganese were found in the Dunbar soil even at the heaviest rate of liming.

On The H-ion Concentration



On The Solubility Of Manganese



On The Solubility Of Iron

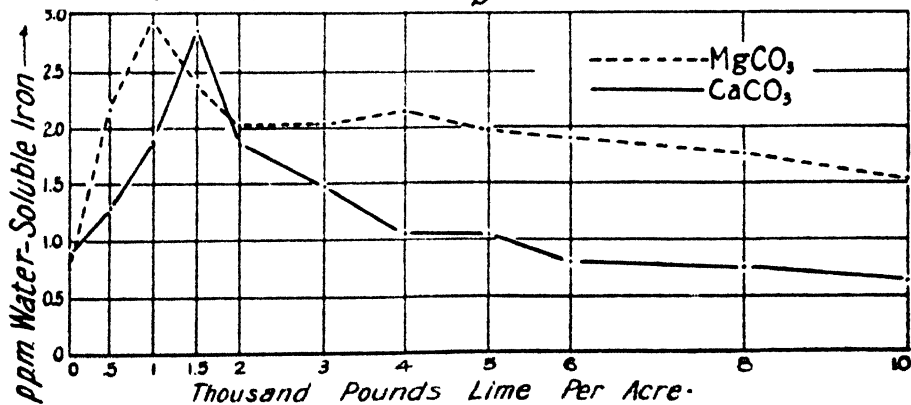


FIG. 2. EFFECT OF APPLICATIONS OF CALCIUM AND MAGNESIUM CARBONATES TO DUNBAR FINE SANDY LOAM

The effects of calcium and magnesium carbonates on the solubility of manganese were identical, as is shown in figure 2.

There was a very sharp increase in the solubility of iron resulting from the addition of lime to the acid soil. As shown in figure 2, by the solubility curves for iron, this reached a maximum with calcium carbonate at the 1,500-pound rate, beyond which there was a decrease in solubility. At the 6,000-pound rate the solubility of iron was the same as that of the unlimed soil and the minimum was reached at the 10,000-pound rate of liming.

With magnesium carbonate the maximum solubility was found with the 1,000-pound equivalent rate of application, with a gradual decrease in solubility from this point with increasing rates of application. The minimum solubility of iron with magnesium carbonate did not, however, become as low as that of unlimed soil.

The solubility curves for iron in the Dunkirk and Dunbar soils have some striking similarities. There is a sharp increase in solubility following the application of lime with maxima reached at less than the maximum pH value. The increase in solubility was better sustained with the greater amounts of magnesium carbonate than with calcium carbonate. This hitherto unnoted behavior of iron as affected by liming is apparently common to soils of widely differing characteristics and involves a principle not evident from the data at hand, although the influence of liming on the solubility of the soil organic matter seems to be a pertinent observation.

EFFECT OF CALCIUM AND MAGNESIUM CARBONATES ON GROWTH AND COMPOSITION OF SOYBEANS

The use of large amounts of dolomitic limestone for tobacco and of both calcitic and dolomitic limestone for peanuts and other crops on some of the dark, sandy, poorly drained soils of the Atlantic Coastal Plain has frequently produced a chlorotic condition of succeeding soybean and other crops. Hence this experiment was made to determine the effect of varying applications of calcium and magnesium carbonates on growth and on the manganese and iron content of soybeans.

On October 10, 1928, gallon glazed pots were set up in triplicate with Dunbar fine sandy loam, as used for solubility determinations, and maintained at 10 per cent moisture until January 8, 1929, when 11 soybean seeds of uniform size and color were planted in each pot. The moisture content of the soil was raised to 20 per cent of the air-dry weight and maintained at this content throughout the growing period.

Notes taken on January 15 indicated no injury to germination from applications of calcium carbonate even at the maximum rate. A delayed germination was noted, however, on the pots receiving high applications of magnesium carbonate. Observations 3 days later showed no injury to seedlings from the use of calcium carbonate, but applications of magnesium carbonate above 4,000 pounds greatly reduced the vitality of the seedlings, those growing in pots receiving the highest amounts dying before the first leaves developed.

On January 20, the beans were thinned to 8 plants in each pot. When these were about 4 weeks old a chlorosis appeared on the lower leaves of all plants receiving applications of calcium carbonate greater than 5,000 pounds an acre, the severity of the chlorosis increasing with the higher rates of application. This symptom, being more pronounced on the lower leaves, did not resemble manganese deficiency chlorosis but may have been due to a magnesium deficiency resulting from the excessive applications of lime. Plate 1, figure 1 illustrates a typical case of this condition.

The decrease in vitality from heavy applications of magnesium carbonate observed when the beans were in the seedling stage increased with the age of the beans, those growing in pots representing treatments 18, 19, and 20 finally dying. As the young leaves developed on these plants they turned brown around the edge, dried, and dropped off. This injury was slight on treatment 15, but increased with magnesium applications.

Because this soil had no fertilization in the field for 15 years, the plants in the pots made limited growth. Therefore when the pods were about half grown and no chlorosis had developed that could be definitely distinguished as due to a deficiency of manganese, two of the series were cut and the roots removed from the soil. One series was fertilized with a solution of one-half gram of ammonium phosphate and one-half gram of potassium phosphate to each pot. The second series was not fertilized. These pots were replanted with soybeans as has been reported previously.

The third series, which was allowed to grow to maturity, gave no indication of a manganese or iron deficiency. The plants of the replanted pots reacted to the calcium and magnesium carbonate treatments in the early stages of growth as did those of the first crop, but the fertilized series made a more vigorous growth than did the unfertilized. When the plants were about half grown, those receiving applications of calcium carbonate above 6,000 pounds on both fertilized and unfertilized series began to show a chlorotic condition of the upper leaves indicative of a manganese deficiency. This diagnosis was confirmed when a green color developed a few days after the chlorotic leaflets were dipped into a 1-1000 solution of manganese sulfate. The magnesium carbonate treatments did not produce a distinct case of chlorosis, probably because the heavy applications were very injurious, no soybeans surviving on treatments 18, 19, and 20. The increased chlorotic condition with increasing calcium carbonate applications is shown in figure 2 of plate 1. Plate 1, figure 3 shows the effect of the magnesium carbonate treatments at the same age.

On June 17, after the pods had formed, the soybeans were cut and sacked separately with the leaves which had fallen from the respective plants. The entire crop from each pot constituted the sample for analysis. The oven-dry weight was obtained by drying for 72 hours at 100°C. The samples were then thoroughly charred at low red heat, moistened with 5 cc. 1-1 nitric acid and evaporated nearly to dryness over a steam bath. The char was then taken up with 1-9 hot nitric acid, transferred to a filter, and washed with 100 cc. of the

hot nitric acid. The residue and filter paper were burned to a white ash and mixed with the filtrate, which was evaporated nearly to dryness before being made up to 100 cc. with 1-9 nitric acid. For the determination of manganese, 25-cc. aliquots were taken, and for iron 10-cc. aliquots. As in previous analytical work, the Stokes and Cain (49) method was used for the determination of iron, and the Willard and Greathouse (50) method for manganese. In table 1 are presented the results obtained for the fertilized series.

TABLE 1
Results with soybeans fertilized and grown on soils treated with CaCO_3 and MgCO_3

POT NUMBER	CaCO_3	DRY WEIGHT OF PLANT	MANGANESE CONTENT OF PLANTS			IRON CONTENT OF PLANTS			Mn REMOVED PER POT	Fe REMOVED PER POT
			First determination	Second determination	Average	First determination	Second determination	Average		
		gm.	p.p.m.	p.p.m.	p.p.m.	p.p.m.	p.p.m.	p.p.m.	mgm.	mgm.
Check	0	7.34	109 0	99 1	104.1	247.6	236.8	242.2	0.764	1.78
1	500	10.83	76 9	73 8	75.4	196.4	205.1	200.8	0.817	2.18
2	1,000	8.41	63 4	56.0	59.7	205 0	230 9	218 0	0.502	1.83
3	1,500	10.26	39.8	41 0	40.4	170 9	173 9	172.4	0.415	1.77
4	2,000	10.01	33 0	29 6	31.3	195 9	185 2	190.6	0.313	1.91
5	3,000	13.47	26 5	25.2	25.9	157 9	138 7	148.3	0.349	2.00
6	4,000	10.64	23.5	24.0	23.8	195 8	208 9	202.4	0.253	2.15
7	5,000	8.61	22 3	20 6	21.5	232.2	207.3	219.8	0.185	1.89
8	6,000	7.31	19 0	18 7	18.9	258.1	223.9	241.0	0.138	1.76
9	8,000	9.07	12 4	11.6	12.0	245 1	232.2	238.7	0.109	2.17
10	10,000	7.51	11 3	8.3	9.8	256 2	242.2	249.2	0.074	1.87
	MgCO_3									
Check	0	3.78	124.5	117.6	121.1	224.2	216.0	220.1	0.458	0.83
11	420	7.37	79.8	67.9	73.9	226.2	212.0	219.1	0.545	1.63
12	840	9.91	59.4	50.5	55.0	213.0	200.2	206.6	0.545	2.05
13	1,260	7.16	48.6	43.0	45.8	249.5	232.8	241.2	0.328	1.73
14	1,680	7.79	36.7	36.7	36.7	256.8	256.8	256.8	0.286	2.00
15	2,520	9.12	31.3	31.3	31.3	210.8	193.9	202.4	0.286	1.85
16	3,360	6.65	25.9	21.6	23.8	254.9	246.5	250.7	0.158	1.67
17	4,200	2.93	24.2	20.0	22.1	232.2	213.3	222.8	0.065	0.65

Pots 18, 19, and 20, receiving 5,040 pounds, 6,720 pounds, and 8,400 pounds of MgCO_3 , respectively, produced no growth.

Liming at moderate rates with both calcium and magnesium carbonates increased the yield. More than the 3,000-pound rate resulted in a slight decrease with the calcium carbonate and distinct evidences of toxicity with the magnesium carbonate. The amount of manganese in the plants decreased very rapidly with increased applications of calcium and magnesium carbonates to the soil. An examination of the data shows that the manganese content of the plants follows a curve very similar to the solubility curve for the soil.

It seems from these results that the manganese content of plants might,

under certain conditions, be as accurate an index of the available manganese in the soil as is the water-soluble manganese in the soil extract. The total amount of manganese removed by each pot decreased as the applications of calcium and magnesium were increased, except in the case of check no. 2, on which the beans made only a very slight growth.

The iron content of the soybeans varied little with the different liming treatments, and no correlation was evident between the iron content of plants

TABLE 2
Results with soybeans unfertilized and grown on soils treated with CaCO_3 and MgCO_3

POT NUMBER	CaCO_3	DRY WEIGHT OF PLANTS	MANGANESE CONTENT OF PLANTS			IRON CONTENT OF PLANTS			Mn REMOVED PER POT	Fe REMOVED PER POT
			First determination	Second determination	Average	First determination	Second determination	Average		
		gm.	p.p.m.	p.p.m.	p.p.m.	p.p.m.	p.p.m.	p.p.m.		
Check	0	4.77	118.1	125.1	121.6	230.3	262.0	246.2	0.580	1.17
1	500	5.89	77.2	81.8	79.5	212.3	226.4	219.4	0.468	1.29
2	1,000	5.51	51.8	58.1	55.0	183.3	193.1	188.2	0.303	1.04
3	1,500	4.47	42.6	46.4	44.5	188.0	203.4	195.7	0.199	0.88
4	2,000	4.63	29.8	31.6	30.7	212.8	216.0	214.4	0.142	0.99
5	3,000	4.81	23.5	23.1	23.3	232.2	225.9	229.1	0.112	1.10
6	4,000	5.68	17.1	17.4	17.3	222.9	227.2	225.1	0.098	1.28
7	5,000	5.78	13.3	14.1	13.7	206.0	226.1	216.1	0.079	1.25
8	6,000	5.11	12.9	13.7	13.3	217.4	217.4	217.4	0.068	1.11
9	8,000	5.31	11.2	12.6	11.9	206.9	221.5	214.2	0.063	1.14
10	10,000	5.26	10.0	9.8	9.9	195.0	218.3	206.7	0.052	1.09
	MgCO_3									
Check	0	2.24	102.0	106.3	104.2	251.5	223.2	237.4	0.233	0.53
11	420	4.48	66.1	68.7	67.4	255.1	279.0	267.1	0.302	1.20
12	840	4.86	50.8	45.7	48.3	207.9	283.9	245.9	0.235	1.20
13	1,260	5.45	34.1	31.5	32.8	188.2	209.7	199.0	0.179	1.09
14	1,680	4.30	21.8	21.9	21.9	255.6	300.1	277.9	0.094	1.20
15	2,520	5.01	17.3	19.0	18.2	257.5	279.2	268.4	0.091	1.35
16	3,360	5.30	12.1	13.8	13.0	235.9	210.8	223.4	0.069	1.18
17	4,200	0.68	0	0	0	262.6	245.1	253.9	0	0.17

Pots 18, 19, and 20, receiving 5,040 pounds, 6,720 pounds, and 8,400 pounds of MgCO_3 , produced no growth.

and calcium or magnesium carbonate applications. The evidence for a manganese deficiency as a cause of the chlorosis, which was shown by the recovery when the chlorotic leaflets were dipped into a manganese solution, is supported by the analytical data, since the chlorotic plants contained as much iron as but less manganese than the green plants.

Results with soybeans grown on the unfertilized series are given in table 2. The yields were only about half as great as in the fertilized series, and no distinct increases were made as a result of liming. The manganese content

of the soybeans representing the various treatments was very similar in respect to the amount absorbed and the effect of liming to those obtained on the fertilized series. No correlation was evident between the iron content of the soybeans and calcium or magnesium carbonate applications. Again it appears that the chlorosis on these soils could not have been due to a deficiency of iron, since the chlorotic plants contained as high a percentage of iron as the green plants.

CAUSE AND CONTROL OF CHLOROSIS OF SOYBEANS ON LIMED SOILS

In connection with the study of the solubility of soil manganese and iron it seemed advisable to examine some of the evidences of response of plants to compounds of these two elements. Soybeans were chosen as test plants because of their known susceptibility to both iron- and manganese-deficiency chlorosis.

Four thousand grams of air-dry Coxville sandy loam soil was weighed in gallon glazed pots and planted with Mammoth Yellow soybean seed. This soil though normally acid had been heavily limed to a pH of 8.4 and had produced chlorotic soybeans in the field. The seedlings were thinned to eight plants and grown under uniform conditions, for three weeks, by which time the apical leaves of all plants were chlorotic. These soybeans were used for the following experiments.

Effect of fertilization on growth and composition of soybeans

Ten cubic centimeters of a one part per hundred solution each of MnSO_4 , FeSO_4 , and MgSO_4 was applied separately to duplicate pots of uniformly chlorotic soybeans. After seven days the soybeans fertilized with MnSO_4 were decidedly greener than those receiving the other treatments. The response to these treatments three weeks after the applications were made is shown in plate 2, figure 1. The plants receiving manganese had developed much larger and greener leaves than those in any of the other pots, whereas the pots receiving magnesia and iron were somewhat greener than the check, indicating a slight response from these materials.

Analyses of the chemicals used for this investigation showed that the manganese sulfate contained considerable iron and that the iron sulfate gave a distinct test for manganese, whereas the magnesium sulfate contained only very slight traces of either. For this reason it was decided to repeat the experiment using purer sources of iron, manganese, and magnesium and including several additional elements.

Using the same cultural methods as before, 10 cc. of a one part per thousand solution of each of manganese sulfate (iron-free), iron sulfate (manganese-free), iron citrate, iron tartrate, copper sulfate, barium chloride, and magnesium sulfate was applied to uniformly chlorotic pots of soybeans. Five days after the treatment the beans treated with manganese sulfate were distinctly greener

than any of the others, which showed no improvement in comparison with the controls. All plants were allowed to grow until pods began to form.

The beans from a check pot and those treated with iron chloride, iron citrate, and manganese sulfate were cut and the manganese and iron contents determined. The methods used for the determination of iron and manganese were the same as those used for the soybean plants, the Stokes and Cain (49) colorimetric method being used for iron determinations and the periodate method of Willard and Greathouse (50) for the determination of manganese. Minor modifications of both were made to adapt them to the materials analysed. Results are presented in table 3.

It will be noted from this table that the plants treated with manganese sulfate made nearly twice as much growth as any of the others. No significant increase in growth was evident from either of the iron treatments over the check. The plants treated with manganese had approximately twice the manganese content of either the check or iron-treated plants, and removed over three times as much from the soil, whereas the iron removed by each pot was

TABLE 3
Manganese and iron contained in soybeans fertilized with manganese and with iron

TREATMENT	DRY WEIGHT OF PLANTS	MANGANESE CONTENT OF PLANTS			IRON CONTENT OF PLANTS			Mn REMOVED PER POT	Fe REMOVED PER POT
		First determination	Second determination	Average	First determination	Second determination	Average		
	gm	p.p.m.	p.p.m.	p.p.m.	p.p.m.	p.p.m.	p.p.m.	mgm.	mgm.
FeCl ₂	12 28	9 0	7.2	8 1	310.0	325 6	317 8	0 100	3 90
FeCit.	13 54	8 4	6.7	7 6	295 6	295 6	295 6	0 103	4 00
MnSO ₄	22 69	14 6	14 5	14 6	196 0	157 0	176 5	0 331	4 01
Check	13 11	8 0	6 9	7 5	305 2	256 1	280 7	0 098	3.68

nearly the same for all treatments. The lower content of iron in the plants receiving manganese seems, therefore, to have been due to the increased growth resulting from the treatment, although there may have been some decrease in the amount of available iron as a result of the manganese addition.

Treatments on the foliage as a corrective of chlorosis

The increased growth and recovery from chlorosis of the plants receiving manganese would establish the fact of a deficiency of that element in the soil if it were not for the possibility of the manganese effect being the result of a secondary reaction in the soil. This possibility was investigated by spraying the foliage of chlorotic plants with 1-1,000 solutions of the same compounds as were added to the soil, precautions being taken in all cases to prevent the spray materials from dripping onto the soil. As shown in plate 2, figure 2, the manganese sulfate as a spray was as effective in overcoming the chlorosis as when applied to the soil and, similarly, no response was noted from any of the

other materials. The evidence appears conclusive that the chlorosis observed is specifically due to manganese deficiency.

A more striking demonstration of this method of showing nutrient deficiencies was made by dipping the center leaflets of chlorotic leaves into the solutions tested as sprays on the foliage. The response is illustrated in plate 3. This method permits of the testing of several elements on a few plants, with untreated leaflets of each leaf serving as controls.

The method is probably limited to those elements required by plants in almost infinitesimal amounts. The corrective effect of manganese solutions applied to the leaves is evident at dilutions as great as one part of manganese sulfate in 100,000 of water (Pl. 4, fig. 1). The recovery of green color by chlorotic leaves following the application of manganese to the foliage was prompt, a response having been noted within 24 hours.

Severely chlorotic plants are characterized as having the lower leaves persistently green while the apical leaves are small and yellow. The function of manganese applied to the leaves appears to be local rather than systemic, for single leaflets, dwarfed by the deficiency, were brought to normal color and size by dipping into a manganese solution (Pl. 4, fig. 2).

SUMMARY

Calcium and magnesium carbonates added to Dunkirk gravelly sandy loam in pots decreased acidity. Both carbonates affected the reaction equally at the lower rates of application, but the magnesium carbonate was less effective at the higher rate.

Liming decreased the solubility of soil manganese, the rate being rapid with the smaller applications. Manganese became relatively insoluble with applications above 5,000 pounds an acre.

The solubility of iron was increased by moderate liming but decreased at the higher rates. The increase was better sustained with magnesium carbonate than with calcium carbonate and the minimum solubility with the former was never less than that in the unlimed soil. The increase in solubility of iron consequent to liming may be due to combination of iron with the soluble organic constituents of the soil dissolved by lime.

With Dunbar fine sandy loam the response to liming was similar to that with Dunkirk soil. The Dunbar soil was the more acid and was less well buffered. Manganese was more soluble but more readily precipitated, although some manganese was soluble at the maximum rate of liming.

Solubility curves for iron had the same characteristics in both soils with maxima at low rates of liming and indicated a greater solubility with magnesium carbonate at the higher rates.

Soybeans grown in pots of the Dunbar soil with calcium or magnesium carbonate and with fertilization responded to moderate liming. Heavy applications of calcium carbonate decreased the yields from the maximum whereas the larger amounts of magnesium carbonate were toxic.

The manganese absorbed by the plants exhibited a curve closely following that of the solubility of manganese in the soil and showing the manganese to be largely precipitated, and absorption decreased at the point of maximum yield.

No significant differences were found in the effects of liming on iron absorption.

Unfertilized plants on this soil made less growth and gave no response to lime other than a change in the color of the foliage, and that due to the toxic effect of heavy applications of magnesium carbonate. The effect of liming on the absorption of manganese and iron was similar to that with the fertilized plants.

A chlorosis of soybeans that had been observed in the field on limed soils was reproduced in pots on a Coxville sandy loam limed to pH 8.4. This chlorosis, typified as being more severe at the apex of the plant, was remedied by applications of manganese sulfate either to the soil or to the chlorotic leaves as a spray. Very dilute solutions were effective when applied directly to the foliage. Iron, magnesium, copper, and barium salts applied to the soil or to the foliage, were without effect on the chlorosis. Diagnosis of manganese-deficiency chlorosis on soybeans is very effectively made by observing the response after one leaflet of a leaf was dipped into a manganese solution.

Manganese-deficiency chlorosis is not a systemic deficiency, as localized applications of manganese salts result in complete recovery of the part both in color and size.

CONCLUSION

The solubility of iron and manganese in soils, the iron and manganese contained in plants, and the response from applications of various salts to the soil and on the foliage of plants indicate that the chlorosis of soybeans grown on certain heavily limed soils is not associated with a deficiency of iron, but is specifically due to a deficiency of manganese.

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PLATE 1

EFFECTS OF CALCIUM AND MAGNESIUM CARBONATES ON SOYBEANS

FIG. 1. A chlorosis of soybeans, severe on the lower leaves, consequent to heavy applications of calcium carbonate.

FIG. 2. Effect of applications of calcium carbonate on growth and color of soybeans.

FIG. 3. Effect of applications of magnesium carbonate on growth and color of soybeans.



FIG. 1



FIG. 2

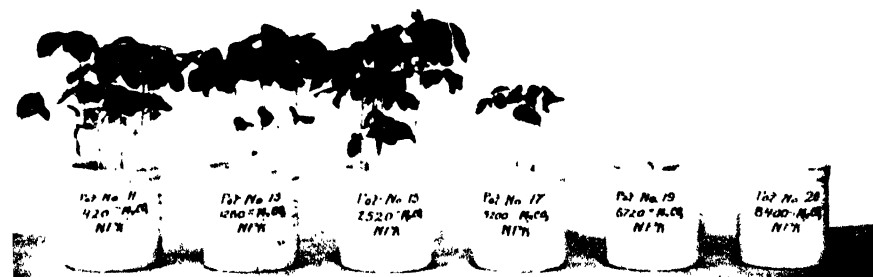


FIG. 3

PLATE 2

EFFECT OF FERTILIZERS AND SPRAYS ON CHLOROTIC SOYBEANS

FIG. 1. Chlorotic Soybeans fertilized with manganese sulfate, iron sulfate, and magnesium sulfate.

FIG. 2. Chlorotic soybeans sprayed with manganese and iron.



FIG. 1



FIG. 2

PLATE 3

EFFECT OF DIPPING THE CENTER LEAFLET OF CHLOROTIC SOYBEAN LEAVES INTO SOLUTIONS
AS INDICATED

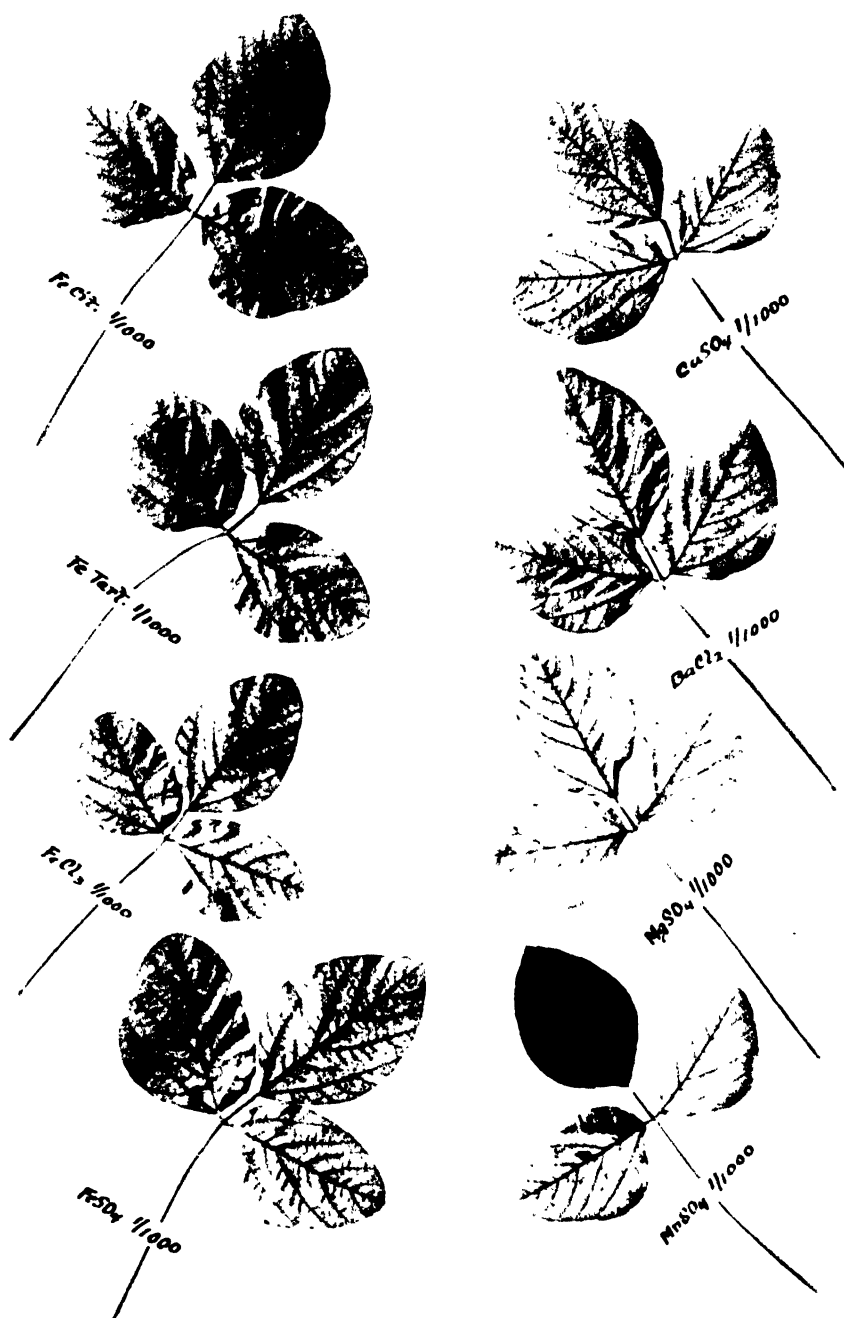


PLATE 4

EFFECT OF MANGANESE SULFATE ON CHLOROTIC SOYBEAN LEAVES

FIG. 1. Effect of dipping the center leaflet of chlorotic soybean leaves into different concentrations of manganese sulfate.

FIG. 2. Response of young chlorotic soybean leaflets to dipping into a 1-1,000 solution of manganese sulfate.



FIG. 1

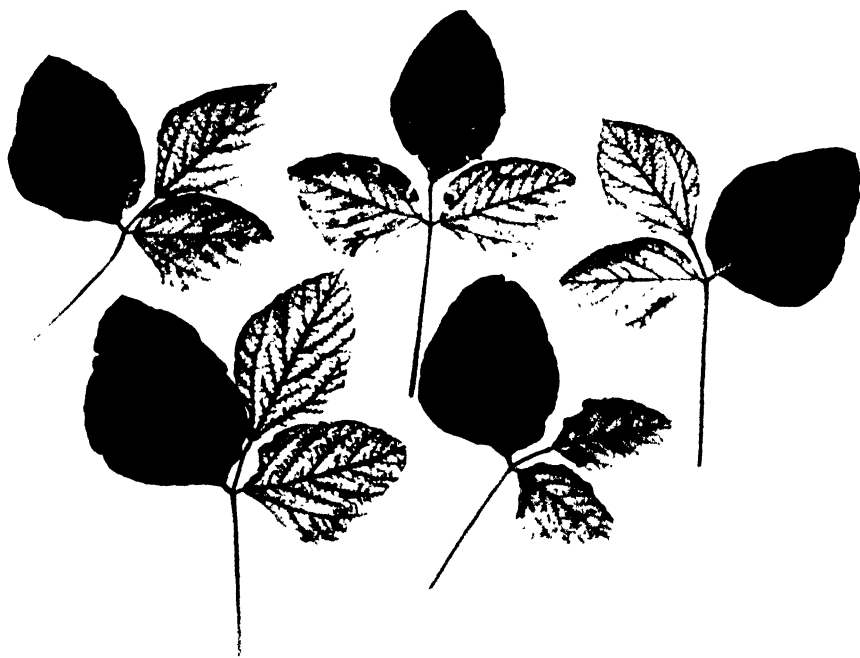


FIG. 2

COMPOSITION OF NATURAL ORGANIC MATERIALS AND THEIR DECOMPOSITION IN THE SOIL: V. DECOMPOSITION OF VARIOUS CHEMICAL CONSTITUENTS IN PLANT MATERIALS, UNDER ANAEROBIC CONDITIONS¹

FLORENCE G. TENNEY AND SELMAN A. WAKSMAN

New Jersey Agricultural Experiment Station

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It has been shown in a previous contribution (6) that the environmental conditions, such as reaction, moisture supply, and aeration, play an important rôle in the decomposition of various complex organic materials of plant origin. These conditions are influential in modifying the nature of the microorganisms taking an active part in the decomposition processes as well as the amount and extent of the organic complex as a whole, and its individual chemical constituents in particular. If the decomposition takes place under aerobic conditions, with a sufficient supply of air and an optimum moisture and temperature, the reduction in the sugars, celluloses, hemicelluloses, fats, and proteins accounts for most of the plant materials that have disappeared. The lignins are much more resistant to decomposition than the polysaccharides and proteins and tend to accumulate; however, this resistance is relative rather than absolute in nature. When the plant material has a low nitrogen content, its decomposition is found to be accompanied by a relative and absolute increase in the protein or organic nitrogen content, due to the synthesizing activities of the microorganisms. These organisms derive their energy principally from the decomposition of the celluloses and hemicelluloses. As a result of this, the addition of inorganic nitrogen salts to plant materials poor in nitrogen and rich in polysaccharides will favor its breakdown by microorganisms.

Even after a considerable period of time (one to two years), a certain residue is left as a result of the decomposition of the plant materials under aerobic conditions at an optimum temperature and moisture. This residue has all the properties of the soil "humus." It is made up chiefly of lignins or modified lignin complexes, of proteins and other organic nitrogenous complexes largely of microbial origin, of hemicelluloses partly of plant and partly of microbial origin, and of various substances in the process of decomposition or resulting from the decomposition of the plant constituents. This "humus" is not in a state of equilibrium but undergoes constant change in chemical composition.

¹ Journal Series paper of the New Jersey Agricultural Experiment Station, department of soil chemistry and bacteriology.

A large part is soluble in alkali, giving a dark pigment, and is reprecipitated by acids, thus having the properties of the "humic acids" of the soil.

To demonstrate the possible differences that may result from the decomposition of organic substances under aerobic and anaerobic conditions, the following illustrations may be cited:

When celluloses are decomposed under aerobic conditions by bacteria, fungi, or actinomyces, they do not yield any organic acids or alcohols, but they result in a more or less complete destruction of the polysaccharide with the liberation of considerable quantities of carbon dioxide; from 20 to 30 per cent of the energy may be utilized for the synthesis of microbial cell substance, rich in organic nitrogenous complexes, such as proteins, and of hemicelluloses and other extra-cellular and intra-cellular products of microbial metabolism (2). Whether the slimy substances produced are synthesized hemicelluloses or intermediary oxycelluloses (11) still remains to be determined. However, when celluloses are decomposed by bacteria under anaerobic conditions, large quantities of organic acids are produced (3, 4, 7).

Under natural aerobic conditions, as in aerated fields or loosely kept manure piles, there is a continuous destruction of organic matter even where large quantities of plant residues are added to the soil every year. However, under anaerobic conditions, as in both acid and neutral peat bogs, there is a continuous accumulation of organic matter, the amount of decomposition being less than the yearly addition of the organic matter by natural vegetation.

Although certain initial processes of decomposition may well go on under anaerobic conditions, as in sewage tanks, for the complete disintegration of the organic residues, proper aeration is required.

In a study made on the decomposition of immature oak leaves under aerobic and anaerobic conditions (10), it was shown that when the leaf material was saturated with water, the celluloses and hemicelluloses were decomposed much more slowly than when the material was under aerobic conditions; the fats and waxes were much more resistant to decomposition and the lignins were preserved almost quantitatively, whereas the protein content of the anaerobic material was considerably greater than that of the aerobic compost because of the greater decomposition of the proteins and losses of the nitrogen in the form of ammonia in the aerobic compost.

These few examples are sufficient to illustrate the differences that may be expected from a comparative study of the decomposition of organic substances under aerobic and anaerobic conditions. They will probably tend to throw some light upon the differences in the nature of organic matter in aerated soil and in peat bogs, if such a difference exists at all.

In order to determine to what extent the decomposition processes are influenced by a change in environmental conditions, a series of experiments were outlined in which the material undergoing decomposition was saturated with water to exclude the free admission of oxygen. Under these conditions, the fungi, most actinomyces, and many aerobic bacteria are excluded, except at the very surface of the material, which is in contact with air. The anaerobic and facultative anaerobic bacteria are largely concerned in the decomposition of the various chemical plant constituents under these conditions. To bring about anaerobic conditions, it is not necessary to exclude the air completely. It is sufficient to cover the plant material undergoing decomposition completely with water, thus reducing the oxygen tension sufficiently to favor the develop-

ment of anaerobic organisms and make conditions unfavorable for the strict aerobic forms.

Five different plant materials, the same as those used in the aerobic decomposition study reported previously (6), were selected for this study; namely, (a) mature corn stalks and leaves, (b) rye straw, (c) mature yellow oak leaves, freshly fallen to the ground, (d) mature alfalfa plants, freshly harvested, (e) the green, growing portion of sphagnum plants, consisting largely of *Sph. acutifolium*. These plant materials were chopped into small pieces and sufficient quantities placed in large glazed earthenware pots to give 200 to 300 gm. of the dry plant substance for each pot. The material was then saturated with distilled water, an excess of free water always being present on the surface of the decomposing mixture. All the pots were then inoculated with a suspension of fresh garden soil, covered with plates, and incubated at 25 to 28°C.

At various intervals of time, the contents of each pot were removed and weighed, the liquid being measured separately; several aliquot portions of the material were then removed from both the solid and liquid portions for the various analyses. Some of the determinations; namely, ammonia, total nitrogen, total dry matter, and water-soluble substances, were made on the fresh portions of material; but the complete analyses were carried out with samples which had previously dried enough to remove the excess of water.

The methods of analyses were those previously described (9), with certain slight modifications. The results were calculated on the basis of the percentage of the residual material as well as on the total original material, allowance being made for the samples removed at different times. In other words, the results are reported in two ways: 1. On the percentage basis of the material left after various periods of decomposition; this enables one to determine the relative changes in the concentration of the various chemical constituents with the progress of decomposition; it also tends to show the progressive formation of the so-called "humus" material, or substances left and those newly formed with the advance of decomposition of various plants. 2. On the basis of the total concentration of the various chemical complexes in the original and in the decomposed material; these data help to visualize even more rapidly the nature of the transformation of the plant material, considered from the point of view of the various chemical complexes that go into its make-up. In dealing with a relatively small quantity of material, the removal of samples at the various periods will involve certain errors in the calculation of the total and relative composition of the residue, unless careful record is kept of the amounts removed at the various periods.

ANAEROBIC DECOMPOSITION OF CORN STALKS, RYE STRAW, AND OAK LEAVES

The corn stalks used in these experiments were rich in water-soluble substances, including reducing sugars and nitrogen compounds. The organic matter as a whole has undergone very rapid decomposition even under anaerobic conditions, but the rate and extent of decomposition are much less than

under aerobic conditions. A comparison of the results presented in tables 1 and 2 with those reported previously (6) on the aerobic decomposition of plant substances, shows that after 27 days, 20.19 per cent of the total material was decomposed under anaerobic conditions, whereas 36.46 per cent disappeared in that same period of time in the aerobic experiments. After 498 days decomposition, 38.18 per cent of the total organic matter was left in the anaerobic

TABLE 1

Chemical composition of corn stalks and decomposed residues, at different stages of decomposition, under anaerobic conditions

On the basis of dry material

CHEMICAL CONSTITUENTS	ORIGINAL CORN	MATERIAL AFTER DAYS OF INCUBATION		
		27	135	498
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
Ether-soluble.....	1.80	1.31	2.63	1.78
Water-soluble.....	14.18	7.89	7.21	4.71
Hemicelluloses.....	17.63	16.76	17.43	16.27
Cellulose.....	29.67	28.51	20.40	26.03
Lignin.....	11.28	14.65	15.96	19.89
Crude protein.....	1.98	3.93	9.63	8.94
(Total N).....	(0.73)	(0.94)	(1.79)	(1.70)

TABLE 2

Total decomposition of the various chemical constituents of corn stalks, under anaerobic conditions

ORGANIC CONSTITUENTS	ORIGINAL MATERIAL	MATERIAL LEFT AFTER DAYS OF DECOMPOSITION					
		27		135		498	
		Total residue	Per cent of original	Total residue	Per cent of original	Total residue	Per cent of original
	<i>gm.</i>	<i>gm.</i>		<i>gm.</i>		<i>gm.</i>	
Total dry material.....	203.00	162.00	79.81	132.50	65.27	77.50	38.18
Ether-soluble fraction.....	3.65	2.13	58.36	3.48	95.57	1.37	37.67
Water-soluble fraction.....	28.71	12.78	44.52	9.54	33.21	3.65	12.70
Hemicelluloses.....	35.79	27.13	75.79	23.09	64.50	12.61	35.22
Cellulose.....	60.24	46.14	76.60	28.53	47.35	20.17	33.48
Lignin.....	22.90	23.73	103.60	21.15	92.34	15.42	67.32
Crude protein.....	4.06	6.38	157.02	12.77	314.41	6.93	170.56

system, and only 22.30 per cent (residual organic matter calculated on the percentage basis of the original fresh plant substance) was left in the aerobic mixture after a shorter period of time, namely after 405 days. In other words, the aerobic decomposition of the same plant substances resulted in a considerably smaller residue than in the anaerobic transformation, as far as the disappearance of the organic material as a whole is concerned.

A comparison of the disappearance of the individual chemical constituents under both sets of conditions, brings out even more striking differences. Of

the three most important groups of chemical complexes in the corn stalks and leaves; namely, those of the hemicelluloses, celluloses, and lignins, the following corresponding quantities were decomposed within 27 days under aerobic conditions: 41.07, 43.56, and 0; whereas under anaerobic conditions, the following corresponding amounts have disappeared in the same period of time: 24.21, 23.40, and 0. In other words, within the first 4 weeks of decomposition, the complex polysaccharides decomposed twice as rapidly when air was freely admitted to the compost. The lignins were resistant to attack by microorganisms under both sets of conditions; however, when decomposition finally set in, it was much slower under the anaerobic conditions than under the

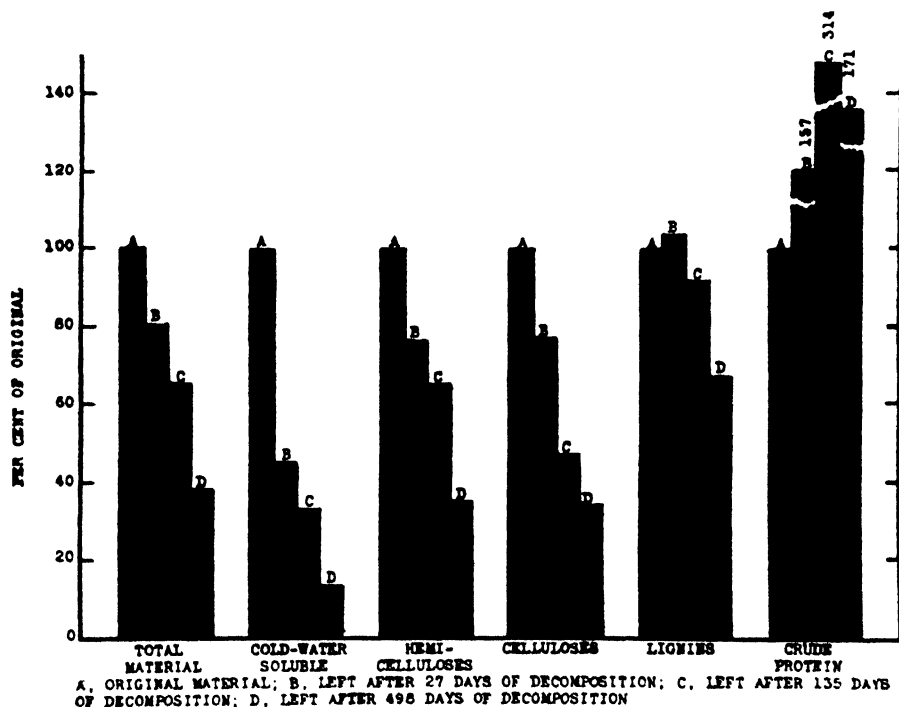


FIG. 1. ANAEROBIC DECOMPOSITION OF CORN STALKS

aerobic: 20.57 per cent of this group of complexes disappeared after 68 days in the aerobic system, but only 7.66 per cent of the lignins were lost after 135 days incubation under the anaerobic conditions; after 405 days, 42.93 per cent of the total original lignin material (including also the variously modified lignin complexes and their derivatives) was left in the aerobic system, whereas 67.32 per cent of this group of substances remained in the anaerobic system, even after 498 days decomposition. In other words, less than a third of the lignins disappeared after a period of nearly a year and a half, under the most favorable temperature conditions. One should mention in this connection that a heavy growth of fungi, actinomycetes, and aerobic bacteria on the surfaces of the liquid

covering the plant material in the anaerobic system does not exclude the possibility that a certain amount of the plant constituents has undergone also an aerobic decomposition.

Figure 1 illustrates the gradual changes that have taken place in the process of transformation of corn stalks when saturated with water, thus excluding a rapid supply of air and favoring the development of anaerobic bacteria, in preference to fungi, actinomycetes, and facultative aerobic bacteria which could

TABLE 3

Chemical composition of rye straw and decomposed residues, at different stages of decomposition, under anaerobic conditions

On basis of dry material

CHEMICAL CONSTITUENTS	ORIGINAL STRAW	MATERIAL AFTER DAYS OF INCUBATION		
		84	163	491
	per cent	per cent	per cent	per cent
Ether-soluble.....	1.84	1.97	1.59	1.47
Water-soluble.....	6.26	3.94	3.40	1.77
Hemicelluloses.....	21.10	20.36	19.48	19.30
Cellulose.....	38.52	35.05	31.31	34.81
Lignin.....	14.63	14.38	16.32	19.28
Crude protein.....	0.81	1.38	3.10	3.82
(Total N).....	(0.28)	(0.56)	(0.61)

TABLE 4

Total decomposition of the various chemical constituents of rye straw, under anaerobic conditions

ORGANIC CONSTITUENTS	ORIGINAL MATERIAL	MATERIAL LEFT AFTER DAYS OF DECOMPOSITION					
		84		163		491	
		Total residue	Per cent of original	Total residue	Per cent of original	Total residue	per cent of original
	gm.	gm.		gm.		gm.	
Total.....	276.90	257.20	92.89	217.30	78.48	181.24	65.43
Ether-soluble.....	5.10	5.05	99.02	3.53	69.12	2.71	53.14
Water-soluble.....	17.34	10.13	58.42	7.43	42.82	3.26	18.80
Hemicelluloses.....	58.42	52.33	89.57	42.19	72.21	34.90	59.74
Cellulose.....	106.66	90.14	84.51	68.03	63.78	62.92	58.99
Lignin.....	40.51	36.99	91.30	35.50	87.63	34.92	86.20
Crude protein.....	2.25	3.59	159.56	6.56	291.56	7.05	313.11

develop only on the surface of the liquid. The water-soluble substances, celluloses, and hemicelluloses have decomposed more rapidly than the total organic matter, whereas the lignins have decomposed much more slowly; the proteins or complex nitrogenous organic substances have accumulated not only relatively but also in actual concentrations when compared with the initial concentration of these complexes in the fresh plant material, taken as 100 per cent.

Results on the rapidity of aerobic and anaerobic decomposition of rye straw, similar to those obtained on the decomposition of corn stalks and leaves, are given in tables 3 and 4. Both the total and the individual chemical constituents of this plant material, with the exception of the cold-water-soluble substances, were found to decompose generally much more slowly than those of the corn stalks. Under aerobic conditions, 17 per cent of the straw decomposed in 66 days and 29 per cent in 143 days, whereas, under anaerobic conditions, 7 and 21.5 per cent were decomposed in 84 and 163 days respectively. The three most important chemical groups; namely, the celluloses, hemicelluloses, and lignins, were found to have decomposed also much more slowly under

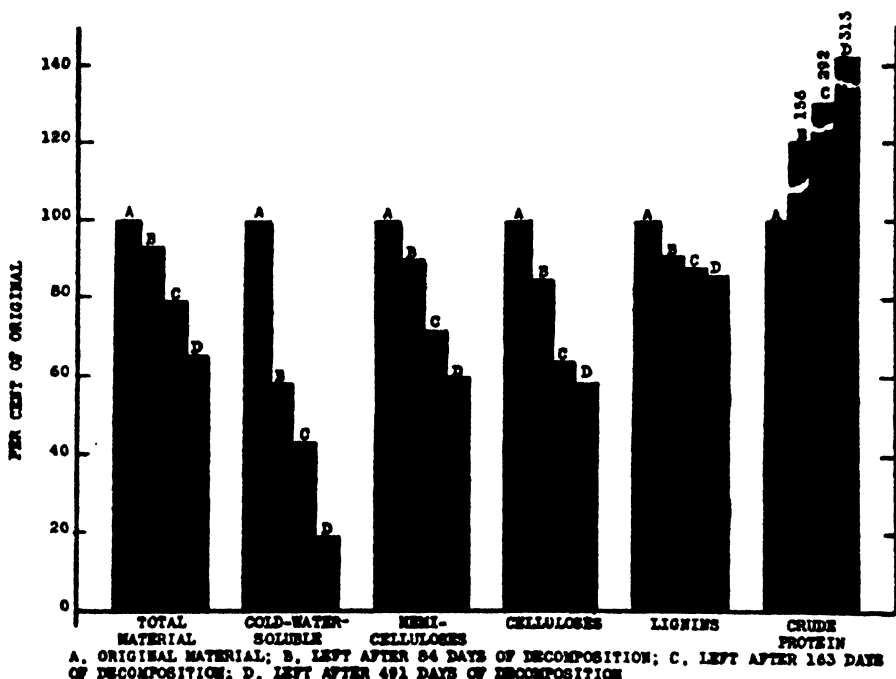


FIG. 2. ANAEROBIC DECOMPOSITION OF RYE STRAW

anaerobic than under aerobic conditions. In the aerobic system, in the absence of nutrient salts, 46.8 per cent of the hemicelluloses, 51.3 per cent of the celluloses, and 20.3 per cent of the lignins disappeared in a period of 386 days; the corresponding quantities of the chemical constituents of this plant substance that have decomposed under anaerobic conditions were 40.3, 41.0, and 13.8 per cent. Figure 2 illustrates graphically the changes that have taken place in the anaerobic decomposition of rye straw. The water-soluble substances were rapidly attacked, these were followed by the celluloses and hemicelluloses. The lignins were very resistant, and the organic nitrogenous compounds rapidly accumulated.

The difference in the rapidity and nature of decomposition of natural plant substances under the two sets of conditions is especially marked in the oak leaves (tables 5 and 6). More than twice as much of the total organic matter decomposed under aerobic conditions as under anaerobic. When the material was properly aerated (about 200 per cent moisture), 42.15 per cent of the total organic matter, 45.09 per cent of the hemicelluloses, 62.56 cellulose, and 15.03

TABLE 5

Chemical composition of oak leaves and decomposed residues, at different stages of decomposition, under anaerobic conditions

On the basis of dry material

CHEMICAL CONSTITUENTS	ORIGINAL LEAVES	MATERIAL AFTER DAYS OF DECOMPOSITION		
		84	163	491
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
Ether-soluble.....	3.71	2.91	3.33	2.53
Water-soluble.....	13.93	5.83	4.49	2.12
Hemicelluloses.....	12.93	14.20	13.37	14.54
Cellulose.....	13.78	13.49	11.75	13.87
Lignin.....	30.30	33.42	35.74	40.32
Crude protein.....	4.25	5.94	6.05	7.29
(Total N).....	(0.82)	(1.00)	(1.03)	(1.17)

TABLE 6

Total decomposition of the various chemical constituents of oak leaves, under anaerobic conditions

ORGANIC CONSTITUENTS	ORIGINAL MATERIAL	MATERIAL LEFT AFTER DAYS OF DECOMPOSITION					
		84		163		491	
		Total residue	Per cent of original	Total residue	Per cent of original	Total residue	Per cent of original
	<i>gm.</i>	<i>gm.</i>		<i>gm.</i>		<i>gm.</i>	
Total.....	222.60	204.39	91.82	199.50	89.70	180.10	80.75
Ether-soluble.....	8.27	5.98	72.31	6.65	80.35	4.56	55.08
Water-soluble.....	31.06	11.89	38.27	8.94	28.78	3.82	12.30
Hemicelluloses.....	28.85	29.00	100.52	27.66	92.41	26.19	90.77
Celluloses.....	30.73	27.58	89.74	23.43	76.25	24.97	81.26
Lignins.....	67.57	68.27	101.04	71.29	105.50	72.62	107.47
Crude proteins.....	4.25	12.17	286.36	12.07	284.00	13.12	308.71

lignin disappeared in 286 days. Under anaerobic conditions, the corresponding quantities that disappeared in 491 days were 19.25, 9.23, 18.76, and 0 per cent. The lignins in the oak leaves did not decompose at all under anaerobic conditions; the hemicelluloses came next in slowness of decomposition.

The accumulation of proteins was in all cases greater under anaerobic than under aerobic conditions. This may be due to the more economic use of the nitrogen, to the smaller loss of ammonia, or to a smaller decomposition of the

synthesized proteins in the anaerobic system. Figure 3 shows the relatively marked preservation of the oak leaves, as a whole, under anaerobic conditions. The most marked phenomenon is the complete lack of decomposition of the lignins. Whether this is because the lignins of the oak leaves are of a different nature from those of corn stalks, since they have a higher fat and wax content, or because of some other factors, still remains to be determined. The much greater resistance of the hemicelluloses than of the celluloses in the case of this plant material also deserves special consideration. This is probably because the chemical composition of the hemicelluloses in oak leaves is quite markedly

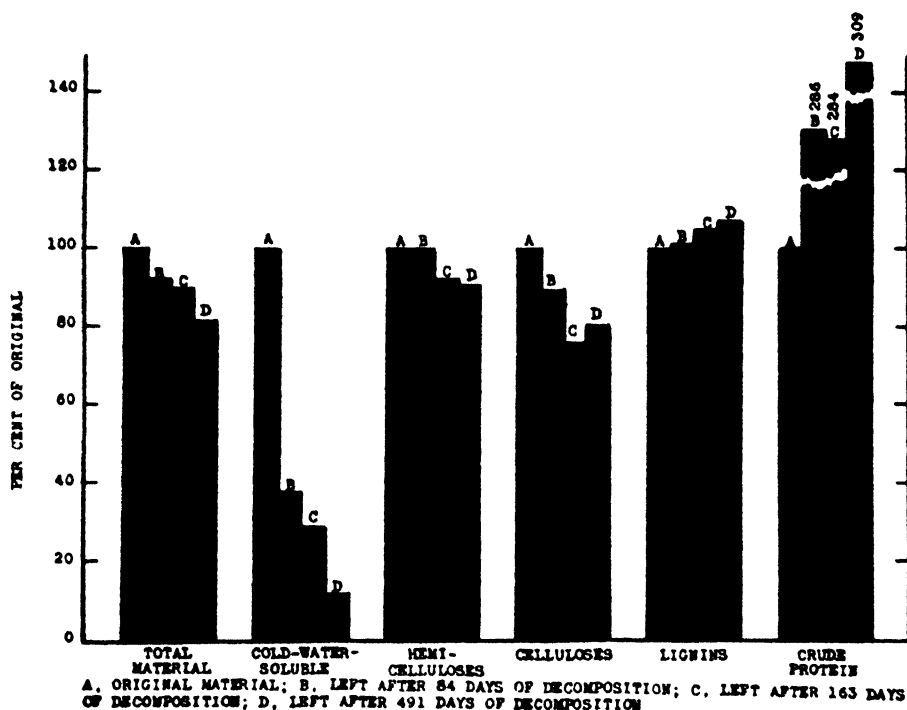


FIG. 3. ANAEROBIC DECOMPOSITION OF OAK LEAVES

different in the tree product from those in the corn residue or the cereal straw, as will be shown in a later publication.

DECOMPOSITION OF ALFALFA PLANTS

The alfalfa plant differed in chemical composition from the other plant materials, especially in its considerably higher nitrogen content. This material also underwent much less decomposition under anaerobic conditions than under aerobic. This is true not only of the total plant substance but also of the celluloses and hemicelluloses. The lignins of the alfalfa plant did not decompose at all in the anaerobic system within a period of 498 days, whereas in the aerobic

system, a marked decomposition of the lignin complexes took place, 40 per cent disappearing within 405 days (tables 7 and 8).

The proteins in the alfalfa underwent an immediate and rapid decomposition under the aerobic conditions; since the amount of nitrogen was greater than that required for synthetic activities by the microorganisms that bring about the decomposition of the celluloses and the hemicelluloses, a large part of the

TABLE 7

Chemical composition of alfalfa and decomposed residues, at different stages of decomposition, under anaerobic conditions

On the basis of dry material

CHEMICAL CONSTITUENTS	ORIGINAL ALFALFA	MATERIAL AFTER DAYS OF INCUBATION		
		27	135	498
	per cent	per cent	per cent	per cent
Ether-soluble.....	2.75	2.37	3.29	2.17
Water-soluble.....	17.24	12.18	13.63	6.74
Hemicelluloses.....	8.52	7.69	7.87	8.65
Cellulose.....	26.71	18.55	17.96	25.03
Lignin.....	10.78	14.89	18.61	23.67
Crude protein.....	8.13	14.91	21.44	8.51
(Total N).....	(2.62)	(3.75)	(4.05)	(1.89)

TABLE 8

Total decomposition of the various chemical constituents of alfalfa, under anaerobic conditions

ORGANIC CONSTITUENTS	ORIGINAL MATERIAL	MATERIAL LEFT AFTER DAYS OF DECOMPOSITION					
		27		135		498	
		Total residue	Per cent of original	Total residue	Per cent of original	Total residue	Per cent of original
	gm.	gm.		gm.		gm.	
Total dry material.....	260.00	190.50	73.27	148.50	57.12	118.00	45.38
Ether-soluble fraction.....	7.15	4.52	63.15	4.90	68.54	2.56	35.73
Water-soluble fraction.....	44.82	23.19	51.74	20.24	45.16	7.95	17.74
Hemicelluloses.....	22.16	14.66	66.13	11.68	52.72	10.20	46.03
Cellulose.....	69.45	35.34	50.89	26.68	38.41	29.54	42.72
Lignin.....	28.03	28.37	101.21	27.64	98.59	27.93	99.64
Crude protein.....	21.14	28.40	134.32	31.86	150.71	10.04	47.47

nitrogen was liberated as ammonia and lost into the atmosphere. Under the anaerobic conditions, however, the nitrogen was largely preserved and only after 135 days of decomposition were any losses observed. This is brought out in table 9. The percentage of nitrogen in the compost at first increases, the total amount of nitrogen remaining constant. There was, however, a drop in the percentage of nitrogen as well as in the total nitrogen in the compost after

135 days of incubation. A rapid decrease in the amount of ammonia also began about that time. Figure 4 shows graphically the transformation of the alfalfa plant constituents when allowed to decompose in a medium saturated with water. The decomposition of the water-soluble constituents, celluloses,

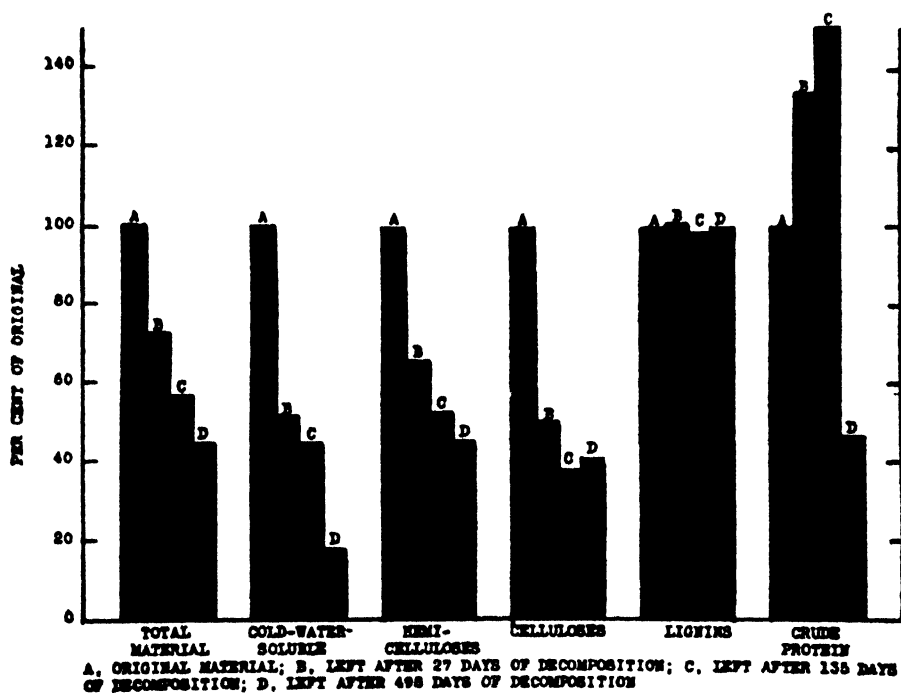


FIG. 4. TRANSFORMATION OF ALFALFA PLANT CONSTITUENTS IN A WATER-SATURATED MEDIUM

TABLE 9
Nitrogen losses in the decomposition of alfalfa under anaerobic conditions

PERIOD OF INCUBATION	N IN COMPOST	AMMONIA N IN COMPOST	RATIO OF $\text{NH}_3\text{-N}$ TO TOTAL N	TOTAL N IN COMPOST
days	per cent	per cent	per cent	gm.
0	2.62	0.04	1.5	6.81
27	3.75	1.07	28.5	7.15
135	4.05	1.09	26.9	6.02
498	1.89	0.22	11.6	2.23

and hemicelluloses is similar in a way to the other plant substances; the complete preservation of the lignins is similar to the processes in the oak leaves, but the transformation of the organic nitrogenous compounds is quite characteristic of this plant substance.

AEROBIC AND ANAEROBIC DECOMPOSITION OF SPHAGNUM PLANTS

The decomposition of the sphagnum plants took place in a manner strikingly different from that of the other plant substances (tables 10 and 11). The decomposition of this material as a whole as well as of some of its chemical con-

TABLE 10

Chemical composition of sphagnum at different stages of decomposition under aerobic and anaerobic conditions

On the basis of dry material

CHEMICAL CONSTITUENTS	ORIGINAL SPHAG- NUM	MATERIAL LEFT AFTER DECOMPOSITION			
		Aerobic		Anaerobic	
		163 days	508 days	163 days	508 days
	per cent	per cent	per cent	per cent	per cent
Ether-soluble.....	1.11	1.23	1.07	1.46	1.50
Water-soluble (cold and hot).....	6.95	1.95	2.39	1.56	0.97
Hemicelluloses.....	27.73	28.40	25.73	28.30	24.70
Cellulose.....	19.21	15.64	16.51	14.58	16.11
Lignin.....	7.33	7.43	8.73	8.26	9.86
Crude protein.....	5.50	4.81	4.41	5.35	3.85
Total N.....	1.10	1.05	0.86	1.06	0.71
NH ₄ -N.....	0.12	0.36	0.23	0.28	0.06

TABLE 11

Total decomposition of the various chemical constituents of sphagnum, under aerobic and anaerobic conditions

ORGANIC CONSTITUENTS	ORIGI- NAL MATE- RIAL	MATERIAL LEFT AFTER DECOMPOSITION							
		Aerobic				Anaerobic			
		163 days		508 days		163 days		508 days	
	gm.	gm.	per cent	gm.	per cent	gm.	per cent	gm.	per cent
Total.....	106.80	94.55	88.53	76.60	71.72	97.90	91.67	96.60	90.45
Ether-soluble.....	1.19	1.16	97.48	0.82	68.91	1.43	120.17	1.44	121.01
Water-soluble (cold and hot).....	7.42	1.88	25.34	1.83	24.59	1.53	20.55	0.94	12.61
Hemicelluloses.....	29.62	26.82	90.53	19.72	66.58	27.70	93.52	23.85	80.52
Cellulose.....	20.54	14.78	72.00	12.64	61.58	14.27	69.55	15.56	75.83
Lignin.....	7.83	7.01	89.53	6.70	85.57	8.08	103.70	9.52	121.58
Crude protein.....	5.87	4.55	77.43	3.40	57.84	5.23	89.10	3.71	63.21
Total N.....	1.17	0.99	84.61	0.66	56.41	1.04	88.89	0.69	58.97
NH ₄ -N.....	0.14	0.34	242.86	0.18	128.57	0.27	192.86	0.06	42.86

stituents was considerably slower than that of any other plant material previously tested; this was especially true under the anaerobic conditions, when less than 10 per cent of the total material disappeared within a period of 508 days. The most interesting phenomenon in the decomposition of the sphagnum plants is the fact that, next to the water-soluble substances, the proteins

or the organic nitrogenous compounds decomposed more rapidly than any other group of chemical constituents, both under aerobic and anaerobic conditions. This is quite contrary to the observations recorded for the other plant materials, when the proteins gradually accumulated, frequently to a concentration several times greater than in the original material. Only in the case of alfalfa, with a very high initial nitrogen content, did the organic nitrogen content diminish after several weeks. In the corn stalks, the initial nitrogen content of 0.73 per cent increased to 1.79 per cent of the residual material, after 135 days under the

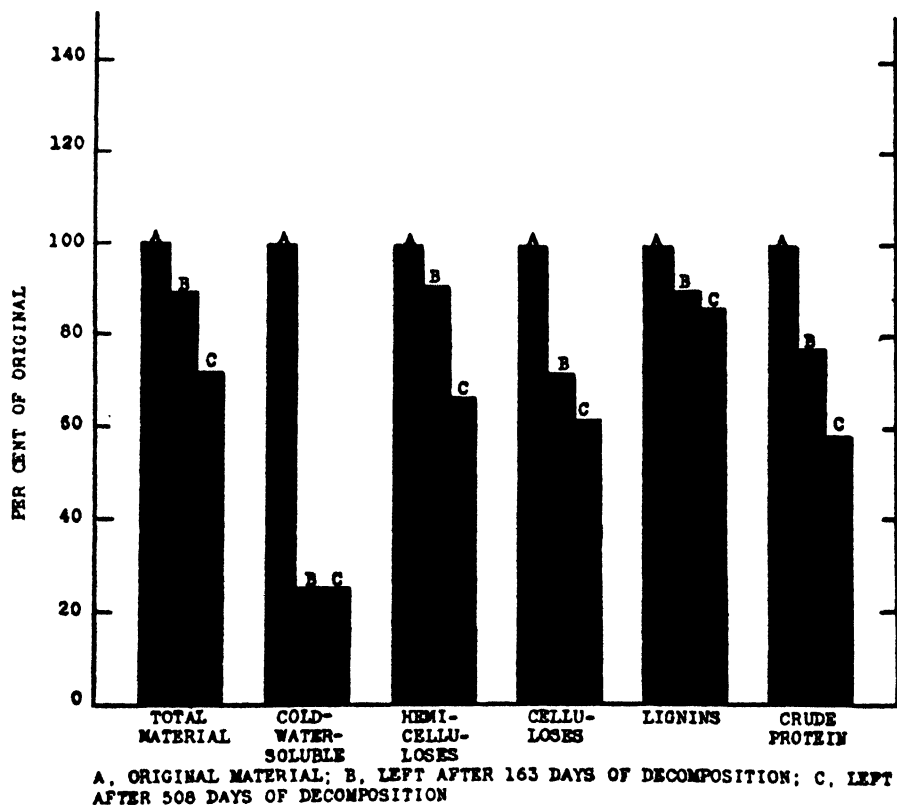


FIG. 5. AEROBIC DECOMPOSITION OF SPHAGNUM PLANTS

anaerobic conditions; in the oak leaves, with a rather limited total decomposition, the increase was from 0.82 to 1.17 per cent; in the sphagnum plants, however, under the same conditions, the nitrogen decreased from 1.17 per cent in the original undecomposed plants to 0.69 per cent in 508 days.

These marked differences in the behavior of the sphagnum plants explain two phenomena commonly observed in peat bogs; namely, the ability of the sphagnum plants to grow continuously in such a poor medium as the sphagnum bog, especially that of the highmoor type; and the formation of large quantities of ammonia as a result of decomposition of sphagnum peat (5, 9). In the

absence of plants that are capable of assimilating the ammonia formed as a result of the rapid decomposition of the nitrogenous compounds, the ammonia will at first accumulate and then gradually be lost into the atmosphere, since nitrifying bacteria are unable to live in such a highly acid medium as the sphagnum bog represents. This is clearly shown in the figures for total and relative quantities of total and ammonia nitrogen (table 11).

Next to the organic nitrogenous compounds in rapidity of decomposition of the various chemical constituents of the sphagnum plants came the celluloses;

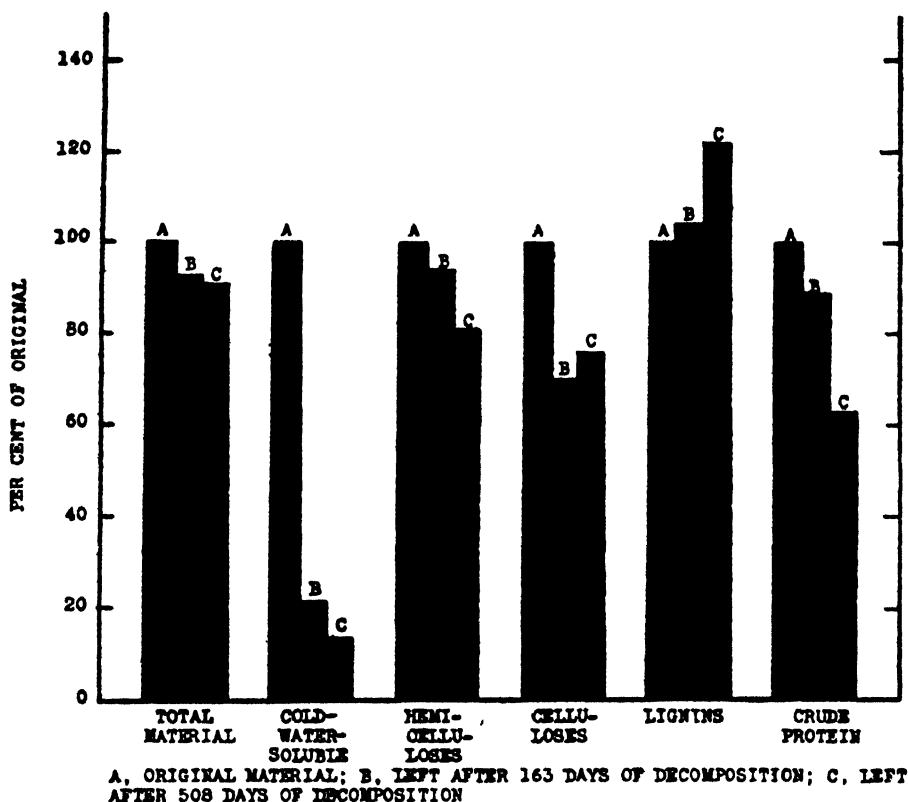


FIG. 6. ANAEROBIC DECOMPOSITION OF SPHAGNUM PLANTS

these were followed by the hemicelluloses. The ether-soluble substances and the lignins of the sphagnum plants were most resistant to decomposition. A certain amount of degradation of these two groups of complexes took place under aerobic conditions; in the anaerobic system, however, these two complexes did not decompose at all and actually increased not only relatively but absolutely. Whether this increase is due to an error in the experimental determination or whether substances of a similar nature were actually synthesized by the microorganisms in the anaerobic system still remains to be seen. Figures 5 and 6 illustrate the processes of transformation of the sphagnum plants

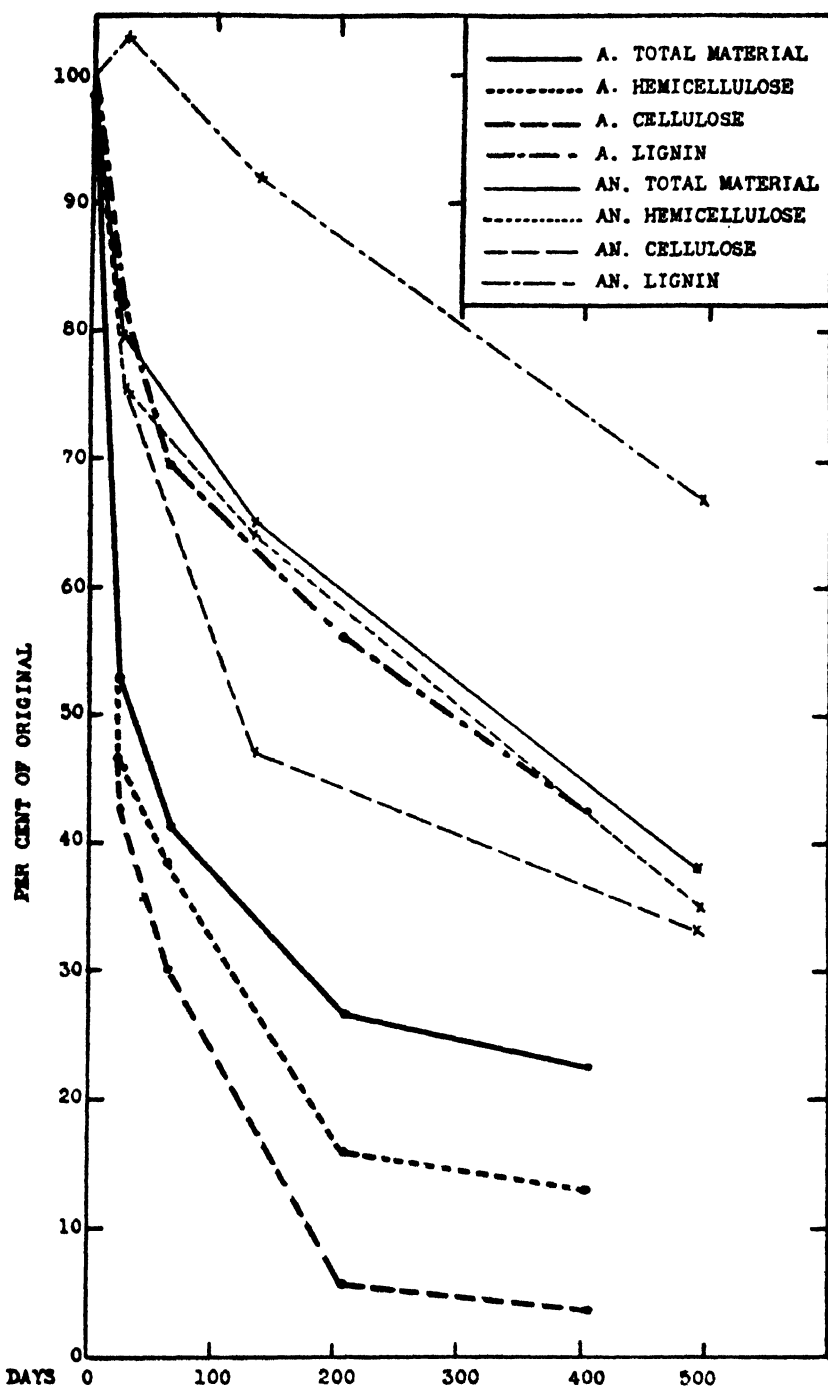


FIG. 7. COMPARISON OF ANAEROBIC (AN.) AND AEROBIC (A.) DECOMPOSITION OF CORN

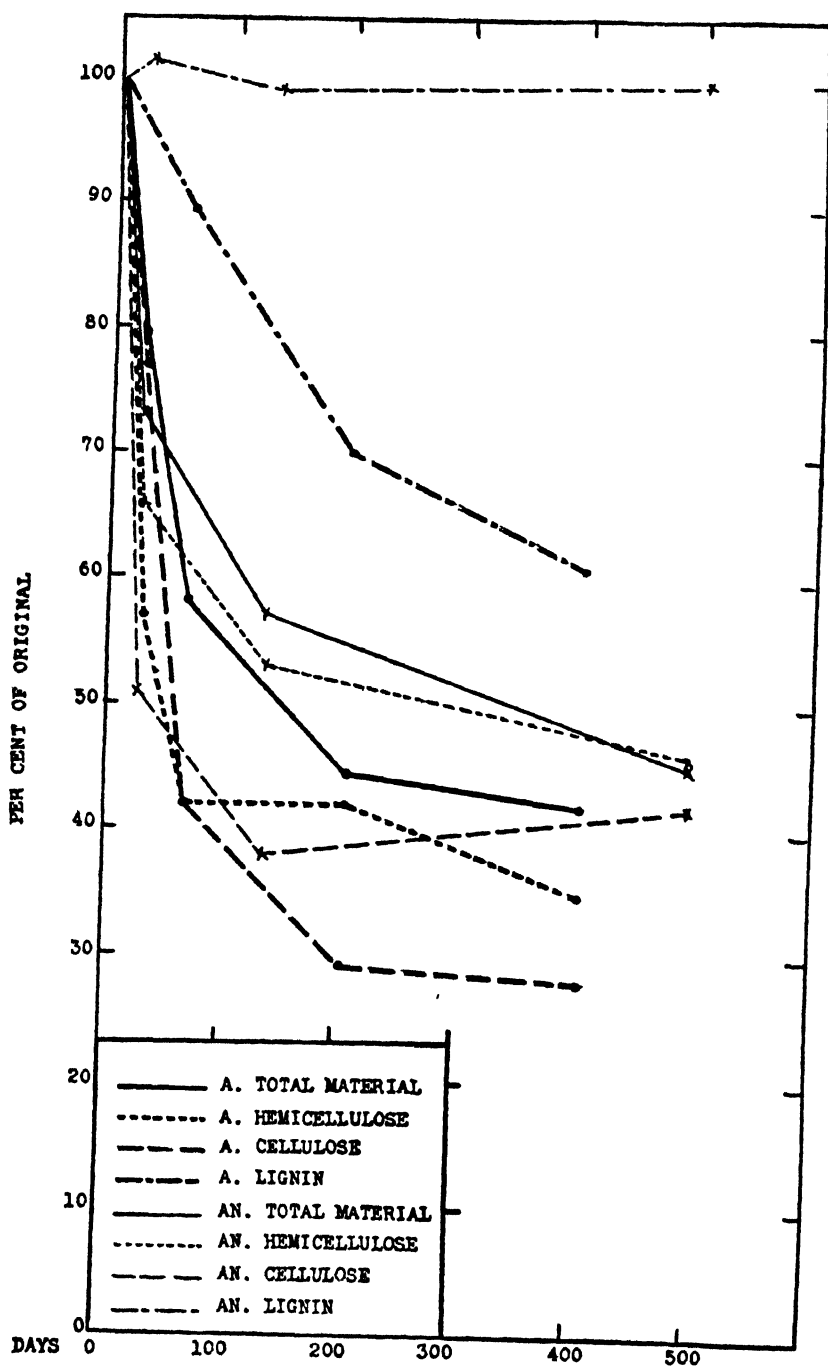


FIG. 8. COMPARISON OF ANAEROBIC (AN.) AND AEROBIC (A.) DECOMPOSITION OF ALFALFA

as a whole and of their various chemical constituents decomposing under aerobic and anaerobic conditions.

To permit a comparison at a glance of the difference in the rapidity of decomposition of two plant materials in a compost to which air was admitted freely (aerobic) and one saturated with water (anaerobic), the results for corn and alfalfa have been redrawn in figures 7 and 8. These figures help one to visualize further, also, the influence of the two sets of conditions upon the decomposition of the various chemical complexes in the two plant materials. Although the total amount of decomposition of the celluloses and hemicelluloses was more rapid than that of the entire material, the decomposition of the lignins was much slower, whereas the rate of accumulation of the organic nitrogenous compounds (proteins) was at first very rapid, then gradually diminished. The curve for protein accumulation is just the reverse of the curves of cellulose and hemicellulose decomposition. They just compensate one another, the former being directly dependent upon the other, the synthesis of the proteins being a direct result of the utilization of the polysaccharides as sources of energy by the microorganisms.

DISCUSSION

Under anaerobic conditions, plant materials as a whole decompose much more slowly than under aerobic conditions. The difference in the rapidity of decomposition of the various chemical constituents is even more striking. This is true especially of the lignins and organic nitrogenous complexes, when compared with the decomposition of the celluloses and hemicelluloses.

These results dealing with the decomposition of various plant materials under anaerobic conditions also explain fully the differences in the chemical composition of lowmoor and highmoor peats or between those peats that have been formed from the decomposition of herbaceous (*Cladium*, *Carex*, *Phragmites*) and of woody plants, on the one hand, and peat that has been formed largely from sphagnum plants, on the other hand. It will be recalled (8, 9) that the organic matter of lowmoor peats is characterized by a high protein and lignin content, and by a low content of celluloses (frequently none), hemicelluloses, and ether-soluble substances. On the other hand, the sphagnum peats are characterized by a low protein and a relatively low lignin content, and by high amounts of celluloses, hemicelluloses, and ether-soluble substances. The studies reported here on the anaerobic decomposition of corn stalks, rye straw, and oak leaves, on the one hand, and of sphagnum, on the other, readily explain these differences in the chemical composition of the two types of peat. The lowmoor peat is made up of plants in which the celluloses and hemicelluloses decompose rapidly while the lignins and proteins accumulate, the latter through the synthesizing agencies of microorganisms that use the polysaccharides as sources of energy. In the decomposition of sphagnum plants, low in lignins, in highmoors, the organic nitrogenous complexes are rapidly attacked; the nitrogen thereby made available cannot be utilized by microorganisms in

the absence of readily available sources of energy, since the polysaccharides of the sphagnum plants are rather resistant to decomposition.

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ANALYTICAL METHODS IN BASE EXCHANGE INVESTIGATIONS ON SOILS

C. J. SCHOLLENBERGER AND F. R. DREIBELBIS

Ohio Agricultural Experiment Station

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It is now the consensus of opinion that in base exchange methods the soil chemist has an effective means for the investigation of many important soil problems. Procedures used by different investigators in this field have varied widely, even though the principles were supposed to be the same throughout. The writers do not intend to enumerate all the methods that have been used in base exchange work, or to enter into any discussion of the merits of each. The procedure that they have used in obtaining data published in another paper (13) will be described and salient points discussed, both as a matter of record which may be of interest to those who study the data presented and in the hope that an account of their experience may be of some help to others who may desire to work along similar lines.

AMMONIUM ACETATE AS A BASE EXCHANGE SALT

A neutral, 2 *M* solution of ammonium acetate was used by Prianishnikov (10) for the estimation of exchangeable potassium in soil; this investigator considered it the most satisfactory salt for the purpose, more especially because it is very easily decomposed by evaporation upon the steam bath, leaving only a slight residue of acetamide which may be volatilized at a slightly higher temperature or destroyed by a digestion with aqua regia. He recommended further study of its use for that purpose, but no one appears to have published anything further on the subject until one of us pointed out the advantageous features of a neutral solution of ammonium acetate for the estimation of all exchangeable cations in soil (11). The salt is almost ideal for the purpose, having numerous theoretical and practical points in its favor, summarized briefly as follows:

1. Ammonium acetate is a neutral salt, and its solutions have pronounced buffer properties around pH 7, as may be seen from the fact that the dissociation constants of both acid and base are practically equal and very small—about 1.8×10^{-5} at 25°C. In theory this is a very important point, because it means that hydrogen displaced from the unsaturated absorption complex of acid soils by the exchange reaction with the salt will be but slightly ionized, hence comparatively inactive. Because the H-ion concentration of the solution is not increased to any considerable extent by the accumulated product of the exchange reaction, replacement of NH_4 from the solution for H in the absorptive complex of the soil can proceed to a maximum.

A single treatment, therefore, should suffice not only for the extraction of all exchangeable cations but for the determination of total absorptive capacity as well. A preliminary treatment with an alkaline reagent has generally been considered essential for complete replacement of exchangeable hydrogen in preparation for the determination of total absorptive capacity. By the substitution of the neutral ammonium acetate for the acid-forming ammonium chloride as the agent for extraction of cations, the necessity for a pretreatment with an alkaline solution is avoided. It has long been known that there is danger of increasing the absorptive capacity of soil by a building up of base exchange complex by the action of strong alkali. Extraction of exchangeable bases by a solution not only neutral at the beginning, but with buffer properties adequate for the maintenance of neutrality has the further important advantage that solution of soil constituents not properly considered exchangeable, but which are dissolved in large amounts by the acid liberated by exchange in solutions of salts of strong acids, is largely or altogether avoided. The determination of "exchangeable" aluminum is an example. With respect to this element, it may be said that aluminum acetate is apparently soluble in a cold solution of ammonium acetate at pH 7, but $\text{Al}(\text{OH})_3$ precipitates when warmed, and does not redissolve again. The acetate solution has no tendency to peptize freshly precipitated $\text{Al}(\text{OH})_3$, as certain other organic ammonium salts have, hence any Al found in an ammonium acetate extract of soil may be presumed to have entered the solution by true exchange.

2. As has been mentioned, ammonium acetate is easily expelled by a simple evaporation during the analytical procedure. The decomposition does not require the use of any additional reagent and is not attended by active evolution of gas to cause loss in spray; separated salt does not creep up the walls of the beaker during evaporation; and the solution boils quietly without bumping.

3. Ammonium acetate is readily soluble in alcohol, permitting the removal by this solvent of the excess salt from the residue of soil, in preparation for the determination of total exchange capacity. Alcohol is superior to water for this purpose, as the tendency to hydrolysis of the ammonium absorption complex is less and alcohol also percolates through the soil more rapidly. It should also be practical to employ ammonium acetate in alcoholic solution as the extracting solution for calcareous soils, in order to minimize attack upon carbonates.

4. A neutral solution of ammonium acetate for base exchange work on soil is readily prepared from inexpensive reagents which may be obtained in high purity or easily purified to any required degree by redistillation.

LEACHING AS A MEANS OF EXTRACTING EXCHANGED CATIONS

Much of the recent work on exchangeable bases of soil has been done by digesting the soil with a portion of the extracting solution, filtering, and washing with more of the same solution. The final washing process is necessary for the removal of an important part of the total exchangeable cations which is in equilibrium with the extracted cations in the portion of solution used for the digestion. In effect, the process is the same as a leaching.

A point in favor of the preliminary treatment with ammonium acetate solution is that better advantage is thereby taken of the buffer properties of the salt. As will be understood from the discussion in a previous paragraph, in leaching an acid soil the exchange of hydrogen from the soil for the cation of the salt results in an accumulation of free acid in the solution. If this liberated acid is diluted by a considerable volume of ammonium acetate solution, the buffer properties of the latter will prevent any considerable increase in the H-ion concentration of the solution. On the other hand, in leaching an acid

soil the first portion of solution to pass through has acted upon successive portions of fresh soil, so that the hydrogen exchange will have been pushed to the limit and the reaction of the first few drops of solution may be as acid as that of the soil. No doubt this action is responsible for some attack upon minerals and solution of non-exchangeable bases, but it does not seem likely that it would ever lead to any great error with an acetate solution. All the work reported by the writers has been done by a leaching procedure, without preliminary digestion. The principal reason for this choice was that the simple leaching can be accomplished with minimum exposure to the atmosphere, which is best avoided on account of the possible effect upon the determination of hydrogen exchanged and ammonium absorbed by the soil. Absorption of CO_2 by the solution or loss of NH_3 from the soil was considered a factor of greater importance than a possible slight increase in amount of some cations extracted from the soil.

If many samples are to be examined, the simple leaching procedure requires less time and labor, as a number of units can be set up and the extraction allowed to proceed without attention. For only one or two determinations, a dispersion of soil in solution with a high speed stirrer (such as a malted milk mixer) followed by filtration and washing on a Buchner funnel, as described by Bayer (2), is more rapid and has been found to give practically identical results so far as extraction of the principal exchangeable bases is concerned. Data obtained by T. C. Green in this laboratory from comparisons of the two procedures on a few samples have indicated, however, that the dispersion method gives considerably lower results than does the leaching method for NH_4 ion absorbed or for total absorptive capacity, so that some acid soils have been indicated to be over 100 per cent saturated with bases. Whether this was due to excessive exposure to the atmosphere during the procedure, possibly resulting in loss of ammonia from the fully saturated soil, was not determined.

There is a possibility that the long time of contact of soil with solution required by a gravity leaching procedure may result in some building up of exchange capacity even at pH 7, and so cause high results for exchanged hydrogen and total absorptive capacity. The possibility of variation in duration of contact with soils differing widely in degree of permeability is a disadvantage of leaching. As in the determination of other "available" soil constituents, the results obtained in base exchange studies depend to a considerable extent upon adherence to a given set of conditions. If the soil is very finely ground, leaching will be very slow, and higher values for most determinations will be obtained. It is a question how much of the increased action noted with ground soils is due to more efficient contact of solution with particles formerly protected in aggregates, how much is due to increase in time of action, and how much is due to exposure of fresh surfaces of undecomposed minerals as the result of the grinding.

Experiments in which 100-gm. portions of acid Wooster silt loam surface soil free from carbonates, air dry, and sieved through 2-mm. mesh, were leached

with 250, 500, 1,000, and 2,000 ml. portions of neutral *N* ammonium acetate have indicated that by the procedure about to be described practically maximum indications for bases exchanged and exchange capacity are obtained by leaching with 500 ml. of the solution. The quantity regularly used has been 750 ml., requiring 18 to 24 hours to percolate through the above described soil. Experience with other soils has indicated that this volume is usually sufficient, with the possible exception of some very heavy clay soils. The quantity of sample used (100 gm.) is larger than that taken by many other investigators in this field. Large samples were considered desirable on account of the low exchange capacity of our soils, and the very small amounts of the alkalis present. A leaching procedure is especially well adapted to work on large samples. Data from analyses of extracts obtained by leaching duplicate 100-gm. samples of 2 mm. acid Wooster silt loam with 750 ml. neutral *N* ammonium acetate and chloride, respectively, are compared in table 1. The most significant differences are in Al dissolved from the soil and in NH_4 absorbed from the solution. Exchangeable H was not determined, but from the data

TABLE 1

Comparison of exchangeable bases in Wooster silt loam as extracted by leaching with neutral ammonium acetate and chloride solutions

Milligram-equivalents per 100 gm. air-dry soil

	Al	Mn	Ca	Mg	K	Na	NH_4 absorbed
	m.e.	m.e.	m.e.	m.e.	m.e.	m.e.	m.e.
Acetate.....	0.16	0.12	1.16	0.32	0.12	0.44	6 76
Chloride.....	2.56	0.28	1.04	0.40	0.12	0.40	3 96

presented a good idea of what it should have amounted to may be formed. The differences between NH_4 absorbed and the sum of bases other than Al extracted in each case indicate that the chloride leaching displaced about 1.8 m.e. H, but the acetate leaching more than twice as much, 4.6 m.e. There is also a considerable difference in the amounts of Mn extracted by the two reagents.

METHODS EMPLOYED

Leaching Solutions

Ammonium acetate sold as a reagent is an acid salt. The reaction of a *N* solution is usually near pH 5, indicating that there is about one mol of free acetic acid to two of the ammonium salt. It is less trouble to prepare a neutral solution from equivalent solutions of acetic acid and ammonia than to attempt to neutralize a solution of the crystals. Three 5-gallon bottles should be at hand. In the first, dilute 2,500 ml. aqua ammonia, sp. gr. 0.9, to 18 liters. Transfer 25 ml. of the solution with a pipette and dilute to 250 ml. in a volume-

tric flask. Transfer an aliquot of this to excess standard HCl and titrate excess acid with 0.1 *N* NaOH and methyl red. Dilute the solution in the bottle to exactly 2 *N*. In a second bottle, dilute 2,100 ml. acetic acid, 99.5 per cent, to 18 liters. Titrate an aliquot of this solution diluted in the same way with the same NaOH and phenolphthalein, and make the solution exactly 2 *N*. In the third bottle, mix 9 liters of each solution. If the solutions were accurately prepared, the mixture will be at pH 7.0. If it is desired to verify this, transfer 100 ml. of the mixture to a 250 ml. beaker, bring to 25° C., add about 0.1 gm. quinhydrone and determine the reaction potentiometrically. If the reaction is not at the desired point, add from a burette the solution required, diluted 1:10, until the reaction is correct. From this titration, calculate the addition to the mixture to bring to the desired reaction. As the comparatively strong acetic acid and ammonia solutions when mixed contract about 25 ml. for each 2 liters, a further dilution to compensate for this will be required to make the solution normal. The writers have considered it desirable to have the leaching solution slightly alkaline in order to ensure maximum absorption of the ammonium ion, and have adjusted it to pH 7.07. Opinions may differ as to the desirability of this, but it is probably not of great importance.

For leaching excess ammonium acetate from the soil prior to the determination of absorbed NH_4 , 80 per cent alcohol has been employed. This was prepared by mixing 16 volumes of 95 per cent ethyl alcohol (so-called "Cologne spirit") with 3 volumes of water. As alcohol of this grade is usually slightly acid, it has been our practice to add sufficient ammonia to cause the color with bromthymol blue to match that of the acetate solution. This neutralization with ammonia is considered desirable, as the removal of NH_4 from the saturated soil by the acid alcohol is thereby prevented and the tendency of the ammonium base absorbent complex to hydrolyze probably reduced. The actual amount of ammonia added is so small that it could scarcely have any noticeable effect in any other way.

Leaching apparatus

Special Pyrex filter tubes 4 cm. inside diameter, 17.5 cm. long including stem 5 cm. long have been employed as containers for the soil during percolation. A 40-mm. perforated porcelain plate rests on three indentations in the glass and is secured and made tight at the edges by means of ceresine wax or "Picein"¹ melted and caused to run around the joint by warming and turning the apparatus. A neatly cut circle of filter paper covers the holes in the plate. The leaching solution is delivered from and received in liter Erlenmeyers, connected with a siphon and an air return tube as shown in figure 1. A thin coating of the cement applied to the lower part of the taper on the no. 9 rubber stopper which closes the top of the percolator is advisable as insurance against

¹ A German preparation obtainable from Wilkens-Anderson Co., Chicago. It is very well adapted to this use, as it melts easily, adheres to glass and porcelain, and is tough, strong, and resistant to alcohol.

any leakage at this point. To fill the percolator, the paper circle is first wetted and spread on the porcelain plate. The dry soil is poured in in several portions, each being worked down somewhat with a spatula, to reduce shrinkage when the solution wets the soil. The apparatus is connected, except the air return tube and cap for escape of air at the top of the percolator. By blowing into the air return tube the siphon is started, and the apparatus then closed. When the ammonium acetate solution has percolated, the apparatus is disconnected, the percolate removed, and 500 ml. alcohol passed through the soil in the same way, to remove ammonium acetate from the soil.

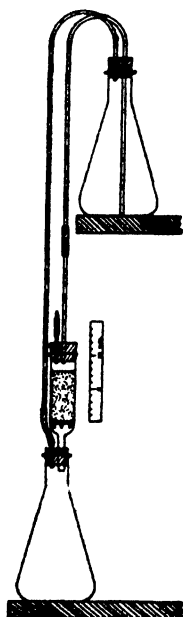


FIG. 1

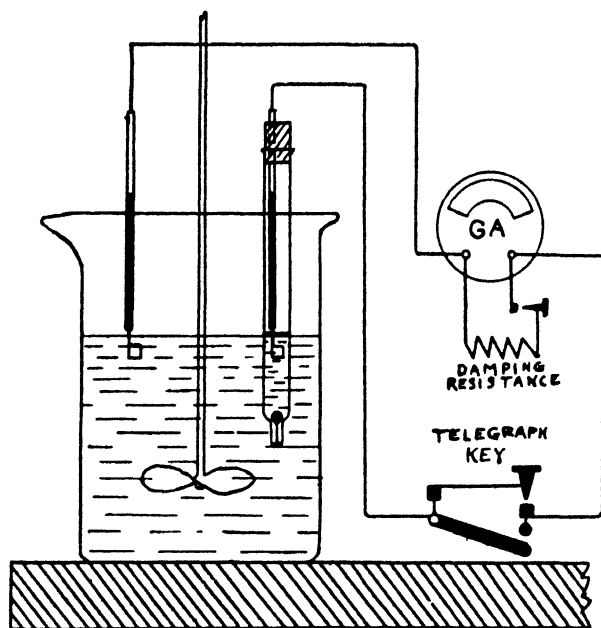


FIG. 2

FIG. 1. APPARATUS FOR LEACHING SOIL

FIG. 2. APPARATUS FOR POTENTIOMETRIC TITRATION

Exchanged hydrogen

The determination of exchanged hydrogen should be carried out as soon as possible after the solution has been removed from the receiving flask. An 800-ml. Pyrex beaker containing the percolate is placed under the apparatus shown in figure 2. The small vessel for the comparison electrode is made from a thin test tube with short tube fused to the bottom and a glass bead with tail ground in with carborundum powder; the ground joint serves as a salt bridge. The two electrodes should be cut from the same piece of platinum foil and fused in glass tubes, with mercury for connection to the wires leading to the galvanometer. The electrodes are prepared for use by heating in $\text{H}_2\text{SO}_4\text{-CrO}_3$,

washing, and ignition in alcohol flame. When used for a succession of determinations on the same day, they may be kept in acetate solution when not in use. Some of the original acetate solution is placed in the comparison vessel, a little quinhydrone added, and the stopper bearing the electrode inserted. This arrangement is supported in the solution so that equality of temperature is ensured. About 0.5 gm. quinhydrone is added to the solution in the beaker and the stirrer started. A quick tap of the key indicates by the deflection of the galvanometer (L. & N. Type R, with 500-ohm coil and provided with damping resistance) whether or not the solution is more acid than it was at the beginning. If acid, 0.2 *N* NH_4OH is added from a burette as rapidly as possible with frequent observations of the effect upon the indications of the galvanometer. As this becomes less, the ammonia is added more slowly, and finally the connection through the galvanometer is made permanent and the spot of light brought to the zero of the scale by additions of drops of ammonia. By standardizing this procedure and working as rapidly as possible, excellent results have been obtained, judged from agreement of duplicate determinations at different times. Titrations of 40-ml. portions of 0.2 *N* acetic acid in 750 ml. neutral acetate have indicated that although there is a plus error averaging 2.2 ml. 0.2 *N* NH_4OH , this is constant to within 0.5 ml. This average correction has been deducted in our work. Fresh solution and quinhydrone must be placed in the comparison electrode vessel for each determination. Following the titration, the beaker with the solution is set on the steam plate to evaporate.

Purification of the residue of salts

The residue from evaporation contains quinhydrone, as well as the acetates of the bases extracted from the soil. Most of the organic matter is volatile without difficulty and ignition in the beaker, similar to the procedure of Bray and Willhite (3), has been applied in the case of a few extracts from limed soils. According to our experience, the ignition in glass has not caused any noticeable contamination with acid-soluble matter, but many small pieces of glass have been detached by the action of the fused salts and the beaker is very likely to break under such severe treatment. With a little extra trouble it would be possible to conduct the final stages of the evaporation and the ignition in a platinum dish with greater safety. The organic matter has usually been destroyed by acid treatment in the beaker as follows:

Add to the residue from evaporation of the acetate extract 15 ml. aqua regia, cover, and evaporate. Add about 15 drops conc. H_2SO_4 and 5 ml. HNO_3 and boil off the acid, keeping the breaker grasped with a Chaddock clamp in constant motion to prevent the acid condensed on the watch glass from dropping on the heated bottom. As soon as the nitric acid has been expelled and heavy fumes of sulfuric acid appear, remove the watch glass and heat the upper part of the beaker as well as the bottom to volatilize organic matter and most of the excess sulfuric acid. Clean the watch glass in the same way and replace it. If the residue is still black, add a little more nitric acid and repeat. This will generally be sufficient. Take up with 15 ml. 5 *N* HCl and 150 ml. water and boil to get CaSO_4 into solution. Filter into a 250-ml. Erlenmeyer, wash, and discard the filter.

Ammonium sulfide precipitation

Neutralize the solution in the flask to methyl red with ammonia, add 5 ml. 5 *N* $(\text{NH}_4)_2\text{S}$, fill to the neck, and stopper. Let stand in a warm place over night. Filter through 9 cm. paper into a 400-ml. beaker and wash rapidly with a cold solution of 10 gm. NH_4Cl and 5 ml. $(\text{NH}_4)_2\text{S}$ in a liter of water, to prevent MnS being reoxidized and entering the filtrate. Roll the paper with the precipitate of MnS and $\text{Al}(\text{OH})_3$ into a ball and preserve in the flask.

Calcium

On account of the wide difference in amount of Ca and Mg in leachings from limed and unlimed soils, different procedures have been used for the determinations. In the case of soils containing little of these elements extracted by ammonium acetate solution, the filtrate from the sulfide precipitate was set on the steam plate and allowed to evaporate to about 75 ml. There should be no precipitate, except a slight deposit of sulfur adherent to the glass, as the result of this evaporation. Acidify with a drop or two of HCl and add dropwise to the boiling solution 5 ml. saturated $(\text{NH}_4)_2\text{C}_2\text{O}_4$. If no precipitate appears, neutralize with ammonia. If the precipitate is heavy, it is probable that more oxalate will be necessary, and 5 ml. more may be added. Digest for about an hour on the steam plate, with occasional addition of a drop of ammonia to keep alkaline. Filter on a small gooch with a paper circle cut to fit, receiving filtrate and first hot water washings in a 150-ml. beaker. When washing is complete, place the crucible with the precipitate in the beaker, add 50 ml. water and 5 ml. conc. H_2SO_4 , heat to 70°C ., and titrate with 0.05 *N* KMnO_4 .

Magnesium

Evaporate the filtrate from the oxalate precipitation to about 60 ml. and cool. Precipitate the Mg by addition of 5 ml. or more *N* $(\text{NH}_4)_3\text{AsO}_4$ followed by dropwise addition, with constant stirring, of conc. NH_4OH in volume equal to one-tenth that of the solution. Set the beaker in a large desiccator jar, or use some other effective means to prevent the escape of the ammonia, and let stand in a cool place until the next day. Filter as in the case of Ca, but wash with 1:10 cold NH_4OH , receiving the filtrate and first washings in a 150-ml. beaker. Drain the beaker thoroughly and draw air through the filter long enough to remove the last drop of wash solution. Place the crucible with the precipitate into the beaker and set aside for an hour to dry, but do not heat to hasten drying. When air dry, all free ammonia will have escaped. Add a small excess of 0.1 *N* HCl and a drop of methyl orange indicator and titrate excess acid, as in Handy's well-known method for Mg (6).

When both Ca and Mg are high, as was the case with our limed soils, the successive precipitation and titration of both without a separation, as originally proposed by Fox (5) but with some change in details in the Mg determination, has been found to be satisfactory. To the filtrate from the sulfide precipitate,

about 300 ml. in a 400 ml. beaker, add a slight excess of HCl and 0.5 gm. oxalic acid. Heat to boiling and precipitate the Ca by very gradual addition of dilute NH_4OH until the odor persists. Let stand 5 minutes, then test for complete precipitation by dropping in a little $(\text{NH}_4)_2\text{C}_2\text{O}_4$ solution. If the precipitation is not complete, it must be made so. When all the Ca is precipitated, add a moderate excess (5 to 15 ml.) of N $(\text{NH}_4)_3\text{AsO}_4$ and ammonia as previously described, after cooling. Filter and wash as if precipitate was of $\text{MgNH}_4\text{AsO}_4$ only, but titrate CaC_2O_4 by suspending the precipitate in 100 to 200 ml. water, adding about 5 per cent volume conc. H_2SO_4 , heating to 70°C . and using 0.2 N KMnO_4 in this case. As soon as this titration is finished, add 5 gm. KI to reduce arsenate to arsenite and set over a burner. Add Na_2SO_3 very carefully² to discharge the iodine color. Continue the heating 15 minutes, adding more sulfite as may be required. Set in water to cool and neutralize most of the acid by careful addition of strong NaOH solution, but leave the solution acid. Add a little starch solution and 0.1 N iodine to color, then destroy this color with a trace of sulfite. Add excess dry NaHCO_3 and more starch solution and titrate to a definite blue color permanent for 5 minutes, with 0.1 N iodine solution, which is conveniently made by dissolving 25 gm. KI in 100 ml. cold water in a 500-ml. volumetric flask, and adding 10 ml. concentrated HCl in 100 ml. water. To this acidified iodide solution add from a pipette 250 ml. 0.2 N KMnO_4 with constant swirling to mix, and make to volume with water. The normality of this solution with respect to Mg will be exactly half that of the KMnO_4 with respect to Ca .

Alkalies

To the filtrate from Mg precipitation and first washings, in a 150- or 250-ml. beaker, is added 12 drops of conc. H_2SO_4 and the solution evaporated as far as possible on the steam plate and still avoiding loss by spitting when the salts begin to separate. The evaporation may be completed and the salts finally dried in an oven at 110°C . At this stage of the work there is considerable danger of loss by creeping over the edge of the beaker, so that it will be well to watch it.

The beaker with the dried residue of salts is set in an electric crucible furnace in a good hood and heated below redness to expel ammonium salts and arsenic. During this ignition, the flame of a burner held in the hand should be directed at intervals upon the exposed rim of the beaker and the watch glass covering it, in order to drive off salts which would otherwise sublime there. When the ammonium salts have been expelled the residue should be nearly white, and aside from insoluble matter (mainly silica) consists of the sulfates of the alkalies. This residue is taken up with a little water and a drop of HCl and boiled for a few seconds to dissolve it. The solution is made alkaline with a drop or two

² Addition of sulfite in excess may cause a yellow color to appear, which is likely to be mistaken for that of iodine. That an excess of sulfite is present will be evident from the odor of the solution.

of ammonia and a little sulfide, and filtered through a small paper into a weighed 100-ml. Pyrex beaker. This solution is evaporated, ignited, and cooled for 15 minutes in the air before being weighed, as a precaution against error from electrification of the glass beaker.

For each centigram of sulfates, 1 ml. PtCl_4 solution (contains 10 gm. Pt a liter) is to be added and the solution evaporated just to dryness. After the residue is cool, excess PtCl_4 is dissolved from the crystals of K_2PtCl_6 with 80 per cent alcohol and the crystals and any insoluble matter in the beaker are transferred to a small Gooch with paper and asbestos, previously dried and weighed. After sufficient washing with the alcohol to remove excess Pt, sulfates are removed by about six washings with half saturated NH_4Cl and the latter removed by a final thorough washing with alcohol. All the filtrates are collected in the same beaker and set aside. The alcohol is allowed to evaporate from the Gooch by standing in the air for several hours, with a final drying at 110°C . before weighing. The potassium salt is removed from the Gooch by washing with hot water and the crucible again dried and weighed. The loss in weight noted between the second and third weighings represents K_2PtCl_6 and is calculated to K, whereas the gain in weight of the third over the first weight represents insoluble matter, which is to be deducted from the weight of alkali sulfates, as determined from the increase in weight of the beaker. The weight of K_2SO_4 calculated from the weight of K_2PtCl_6 found is further deducted, leaving the weight of Na_2SO_4 .

All the errors of the analysis fall upon the sodium determination, hence it is well that the analyst satisfy himself that the previous separations were complete. Manganese and magnesium are most likely to escape precipitation at the proper point; the presence of the former in the alkali sulfates will be shown by a brown color, but the latter is not so easily detected. If these were present, they will remain in the solution from which excess Pt was precipitated by NH_4Cl . The Pt salt is filtered off and washed with a little more NH_4Cl solution to dissolve sulfates. The clear alcoholic filtrate is tested first with a little sulfide for Mn, then with oxalate for Ca, and finally with arsenate for Mg. The separations from this alcohol solution are rapid and the testing does not require much time. If appreciable quantities are found, determination as sulfate or calculation to that form and correction of the weight of alkali sulfates by the proper deduction are necessary.

Aluminum

The filters containing the main sulfide precipitate as well as any that may have been recovered while working with the alkali sulfates, are removed from the flask and ignited in a platinum crucible. The ash is fused with KHSO_4 at red heat and the melt dissolved. In the meantime 5 ml. conc. HNO_3 has been evaporated in the flask to remove the chloride and the flask then rinsed with a little HNO_3 and water. This solution and that of the bisulfate melt are united in a 200-ml. volumetric flask and made to volume. The solution is

filtered through a dry paper, and half of it taken for the determination of Al by an adaptation of Burgess' (4) method; to the 100-ml. aliquot in a 150-ml. beaker are added a drop of alizarin-S indicator and ammonia to a brownish red tint, indicating pH 5. Then are added successively 2 ml. N $(\text{NH}_4)_2\text{HPO}_4$, 1 ml. N 5 HCl to clear, and 2.5 ml. N $\text{Na}_2\text{S}_2\text{O}_3$; and this mixture is boiled slowly while covered 15 minutes. After the addition of 5 ml. 5 N $\text{NH}_4\text{C}_2\text{H}_3\text{O}_2$ at pH 5, the boiling is to be continued for 15 minutes longer, then filtered and washed with hot water. If much Mn is present, it will be necessary to dissolve the AlPO_4 from the precipitate with hot dilute HCl, catching the solution in the same beaker and repeating the procedure in order to get a good separation. The precipitate of AlPO_4 and S is finally ignited in a porcelain crucible and weighed as AlPO_4 .

Manganese

The remaining 100 ml. of solution of bisulfate fusion, or an aliquot if Mn is high, is used for this determination. It is transferred to a 250-ml. beaker and 5 ml. conc. HNO_3 and 25 ml. AgNO_3 (1 2 per cent solution) are added, followed by 1 gm. $(\text{NH}_4)_2\text{S}_2\text{O}_8$. The beaker is set upon the steam plate to heat slowly to 90°C ., at which point the maximum permanganate color should be developed. The solution is cooled quickly, Ag precipitated by the addition of 25 ml. NaCl (0.4 per cent solution), and without filtering titrated to disappearance of color with standard Na_2HAsO_3 solution as described by Scott (14).

Exchangeable Ammonium

A procedure similar to that described by Merkel (8) was employed for the estimation of this constituent; 100 gm. dry soil, as used for the ammonium acetate leaching, is weighed out and transferred to a 500-ml. Erlenmeyer. The flask is filled with 0.1 N HCl and shaken at short intervals for 5 minutes, then the suspension filtered on a 10-cm. Buchner. The filtrate is transferred to a liter volumetric flask, and the soil washed with more of the acid until the total volume is 1 liter. An aliquot of 500 ml. is transferred to a copper flask, a slight excess of alkali added, and the ammonia distilled into excess standard H_2SO_4 , and titrated with 0.1 N NaOH and methyl red indicator. In some instances, the determinations by this method were checked by the method of McLean and Robinson (9), employing a solution of NaCl for extracting NH_4 from the fresh soil and distilling off NH_3 as described in the foregoing. As good agreement was obtained, either method seems equally reliable.

Ammonium absorbed

The method described by Kelley and Brown (7), with slight changes, has been used for the determination of NH_4 absorbed from ammonium acetate by the soil, or total absorptive capacity. Following the leaching with alcohol to remove acetate, as much of the alcohol as possible is drawn from the soil in the percolator by application of suction, but care being taken not to dry the

soil, which would result in the loss of considerable absorbed NH_3 . The moist soil is turned from the percolator into a weighed beaker with cover, and after thorough mixing with a spatula the weight of damp soil is determined. One fourth of the soil is transferred to a copper flask, 400 ml. water with a few drops of capryl alcohol and mineral oil added to reduce foaming during distillation, and 50 ml. strong NaOH added, and the NH_3 distilled slowly for 45 minutes. A similar determination on 25 gm. of the original soil has been applied as a blank. It would have been better to have removed the small amount of exchangeable NH_4 from the blank sample by extraction with acid or salt solution although the error introduced by this neglect was insignificant and can be corrected by other data obtained. The blank is quite large, because of decomposition by the drastic treatment of organic matter containing nitrogen. In our experience it has been quite constant for a particular soil, and this method is considered satisfactory for the determination if carried out with some precaution to ensure uniform distillation period for all samples.

Correction for carbonates dissolved

The residue of percolated soil after removal of the portion for determination of NH_4 absorbed may be dried and ground for determination of carbonate dissolved from a calcareous soil during the leaching process, comparing the data so obtained with that from examination of the original sample. The apparatus we have used for the determination of carbonates in soil by boiling with 1:50 HCl *in vacuo* has been described (1), also the absorption of CO_2 evolved in excess $\text{Ba}(\text{OH})_2$ and titration (12).

Blanks

In consequence of the large volume of solution which is used for the extraction of the exchangeable bases from the soil, appreciable amounts of impurities are introduced, even when the best reagents obtainable are used. In our experience, a small amount of calcium and a considerable amount of sodium have invariably been found in blank determinations with the regular procedure, but without soil. It has been noticed that these blanks are larger when old aqua ammonia has been used for the preparation of the ammonium acetate solution for leaching the soil, or when the acetate solution had been kept for a considerable time in a bottle of common glass.

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STUDIES OF SOILS IN THE PLASTIC STATE

G. B. BODMAN AND M. TAMACHI¹

University of California

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The work of Atterberg and Johannson upon soil consistency published between 1911 and 1916 (1, 2, 10 and numerous other papers) has lately received attention in this country and in England. Atterberg's methods have been reviewed at some length by Russel and Wehr (15). These authors gave detailed descriptions of their adaptations of the Atterberg methods, and determined the Atterberg numbers for the various consistencies of a number of Nebraskan soils. Bayer more recently has given the effects produced by controlled base replacement upon some of the Atterberg numbers (3). The "lower liquid limit" and "lower plastic limit" are commonly determined in the preliminary examination of subgrade material for road construction in the Bureau of Public Roads of the U. S. Department of Agriculture. Wintermeyer (20) has described the methods for obtaining these values. Their significance from the standpoint of the identification and evaluation of soils for construction material has been discussed by Terzaghi (18). As reported by Davis (6) some of the Atterberg numbers have been determined for certain soils collected by the U. S. Bureau of Chemistry and Soils. Haines, working at the Rothamsted station, examined soil cohesion and the pressure of fluidity for certain clay separates, ball clays, and a few soils (9) as part of an investigation into the mechanical properties concerned in cultivation. Methods very similar to those of Atterberg and Johannson were used for the cohesion measurements. A few years after the appearance of the first (1, 2) of Atterberg's papers on soil consistency, Kinnison of the U. S. Bureau of Standards compared the order of relative plasticity as determined by the Atterberg method, with that obtained by some of the then used methods for measuring plasticity in America, for a variety of shales, kaolins, and clays (11). He concluded that no single method is sufficient to classify clays as to their plasticity, and that each included some results out of harmony with the observed facts as recorded from common experience with the clays studied. He considered that the most reliable rating for the series was to be obtained from the product of the plasticity number, the water of plasticity, and the water vapor adsorbed over 10 per cent sulfuric acid.

DESCRIPTION OF SOILS USED

It was the purpose of the present investigation to examine the relation of the moisture content at the plastic consistency to some of the other properties of a fairly large number of Californian surface soils. Forty-three soils, members of 23 soil series, and 1 unnamed lacustral soil, were used. In addition 10 soils from India, members of 8 soil series, were included in the study. The Californian soils consist of primary, or residual, and secondary soils. The primary soils have developed from parent material which includes basic and meta-

¹ Assistant professor of soil technology, University of California, and professor of agricultural engineering, Kyushu Imperial University, Fukuoka, Japan, respectively.

TABLE 1
Description of soils used
(After Shaw)

NUMBER	DIVISION	FAMILY	STAGE	GROUP	SERIES AND TYPE	AREA
<i>Californian soils</i>						
<i>Mineral soils—primary</i>						
1. Without lime accumulations in the soil profile						
1	B	Claypan	Semimature	Dark	Climax clay adobe	Ukiah
2	B	Claypan	Semimature	Dark	Climax clay	Healdsburg
3	B	Claypan	Semimature	Brown	Olympic clay adobe	Bay Region
4*	B	Claypan	Semimature	Red	Aiken stony loam	Grass Valley
A(1)*	B	Claypan	Semimature	Red	Aiken clay loam	Placerville
2. With lime accumulations in the soil profile						
5	C	Claypan	Semimature	Dark	Diablo clay loam adobe	Santa Maria
<i>Mineral soils—secondary</i>						
1. Without lime accumulations in the soil profile						
6*	A	Iron-silica hardpan	Mature	Red	San Joaquin sandy loam	Sacramento Valley
7	B	Unknown	Recent	Dark	Pit clay adobe	Big Valley
8	B	Unknown	Recent	Dark	Pit clay loam	Big Valley
9	B	Unknown	Recent	Brown	Vina clay adobe	Big Valley
10*	B	Unknown	Recent	Brown	Vina fine sandy loam	Chico
11*	B	Unknown	Recent	Brown	Vina fine sandy loam	Chico
12	B	Unknown	Immature	Brown	Gould clay adobe	Big Valley
13	B	Unknown	Recent	Brown	Buntingville clay loam	Big Valley
14	B	Unknown	Recent	Brown	Buntingville clay loam	Big Valley
15	C	Claypan	Recent	Brown	Wapato silty clay loam	Willits

16	C	Claypan	Recent	Dark	Dublin clay	Ukiah
17	C	Claypan	Recent	Dark	Dublin clay adobe	Bay Region
18	C	Claypan	Recent	Brown	Yolo silty clay loam	Ukiah
Yc(1)	C	Claypan	Recent	Brown	Yolo clay	Davis
Ycl(1)	C	Claypan	Recent	Brown	Yolo clay loam	Davis
19	E	Claypan	Recent	Brown	Bayside silt loam	Eureka
20	E	Claypan	Recent	Gray	Coquille silty clay loam	Eureka

2. With lime accumulations in the soil profile						
21	B	Lime and lime-iron hardpan	Mature	Dark	Gazelle silty clay loam	Big Valley
22*	B	Lime and lime-iron hardpan	Young	Brown	Nord fine sandy loam	Chico
23	C	Lime hardpan	Semimature	Dark	Montezuma clay adobe	Santa Maria
24	C	Lime hardpan	Young	Brown	Willows clay	Cortena
25*	C	Lime hardpan	Young	Brown	Willows clay	Cortena
26	E	Lime hardpan	Semimature	Dark	Stockton clay adobe	Biggs
27*	E	Lime hardpan	Semimature	Dark	Stockton clay adobe	Biggs
28*	E	Lime hardpan	Semimature	Dark	Stockton clay adobe	Biggs
29	E	Lime hardpan	Semimature	Dark	Stockton clay adobe	Biggs
30*	E	Lime hardpan	Semimature	Dark	Stockton clay adobe	Biggs
31	E	Lime hardpan	Semimature	Dark	Stockton clay adobe	Biggs
32*	E	Lime hardpan	Semimature	Dark	Stockton clay adobe	Biggs
33	E	Lime hardpan	Immature	Gray	Stockton clay adobe	Biggs
34	E	Lime hardpan	Immature	Gray	Stockton clay adobe	Biggs
35	E	Lime hardpan	Immature	Gray	Lahontan silty clay loam	Honey Lake
36	E	Lime hardpan	Immature	Gray	Lahontan clay adobe	Honey Lake
37	E	Lime hardpan	Recent	Brown	Lahontan clay	Honey Lake
38	E	Lime hardpan	Recent	Brown	Holtville silty clay loam	Brawley
39	E	Lime hardpan	Recent	Brown	Imperial clay	Brawley
40	E	Lime hardpan	Recent	Brown	Imperial silty clay loam	El Centro
					Lacustral clay	Brawley
						Bay Region

TABLE 1—*Concluded*

NUMBER	DIVISION	FAMILY	STAGE	GROUP	SERIES AND TYPE	AREA
<i>Indian soils</i>						
Mineral soils						
41					Vindhyan loam	Shivpuri
42					Shivpuri loam	Guna Rd.
43*					Gwalior silt loam	C. E. Farm, Gwalior
44					Shivpuri loam	Gujarda
45*					Vindhyan loam	Shivpuri
46					Malwa clay loam	Tajpur Ujjain
47					Malwa clay loam	C. Farm Ujjain
48					Sipra clay loam	Dhondarka
49					Singanwas clay loam	
50					Nahri clay loam	Fatchpur

* Not surface soils.

*Explanation*1. *Division* refers to geological origin of parent material.

A—Soils composed of materials from disintegrated and decomposed igneous and metamorphosed igneous rocks high in quartz.

B—Soils composed of materials from disintegrated and decomposed igneous and metamorphosed igneous rocks low in quartz.

C—Soils composed of materials from disintegrated and decomposed sandstone and shale rock sources.

E—Soils composed of materials from disintegrated and decomposed mixed or undetermined rock sources.

2. *The Family* is based on the character of the profile shown by the fully weathered mature soil.3. *The Stage* is based on the maturity of the soil.4. *The Group* is based on the color of the surface soil.5. The term *Series* has its usual significance.

6. The textural description is based on the surface soil.

morphosed igneous rocks, sandstones, and shales. The secondary soils have developed from unconsolidated sediments derived from a still wider variety of rocks.

They were collected at altitudes varying between 200 feet below sea-level in

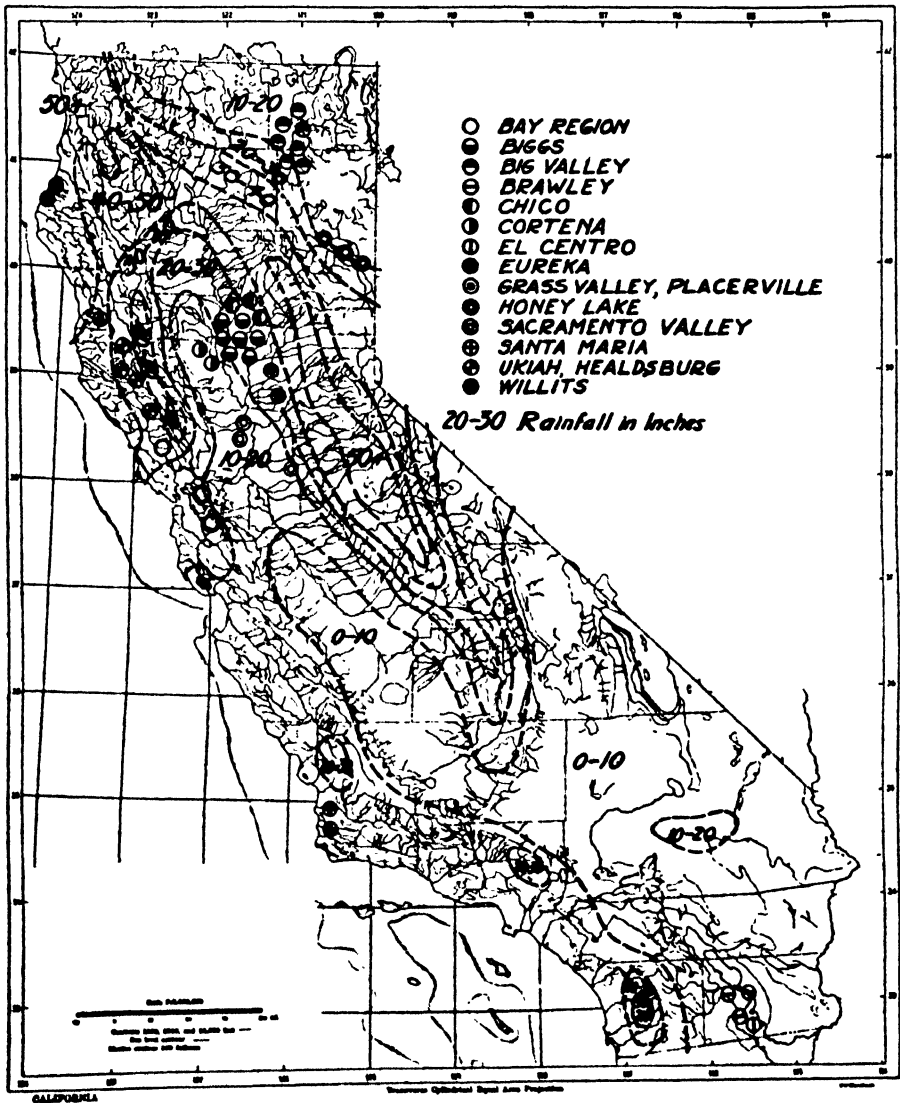


FIG. 1. OUTLINE MAP OF CALIFORNIA SHOWING LOCATION OF SOILS

Imperial Valley and 4,300 feet above sea-level in Big Valley. They represent climatic conditions with annual precipitations of from less than 4 to over 50 inches, and temperatures which are practically frost free to those having frosts during 10 months of the year. They were taken from the collection of soil

samples made by the University of California and the U. S. Dept. Agriculture Bureau of Chemistry and Soils in the course of the soil survey of California. This collection includes representative profile sets of all the soil series established in California. The classification of the soils used is given in table 1, and their geographic distribution is indicated in figure 1. The distribution of the average annual rainfall in California (19, p. 3) is also given in the figure.

The descriptions are based on "The Basis of Classification and Key to the Soils of California" by Shaw (17). The Indian soils are from the general vicinity of Allahabad in a region of high annual precipitation and high mean temperature. They were collected by Pendleton and are described more fully in the soil survey reports of the Gwalior, Mandsaur, Ujjain, and Shivpuri areas, India (13).

All of the soils had been preserved in glass jars for at least one year, and in some cases for as long as 15 years, and were air-dry. Each was prepared before use by being passed through a 1 mm. screen, followed by thorough mixing. The Indian soils had been sterilized upon entrance to this country five years previously. Except as indicated, all were surface soils.

THE PLASTIC CONSISTENCY

Plasticity, or the plastic consistency, may be defined as a property of solids by virtue of which they hold their shape permanently under the action of small shearing stresses, but are readily deformed, worked, or molded, without cracking, under somewhat larger pressures. The main part of the definition is according to Bingham (4, p. 216). Mellor (12, p. 354) adds "without cracking" to an essentially similar but independent definition. It is obviously possible to exclude the very sandy soils from those which exhibit plasticity under ordinary conditions to an even moderate degree. Accordingly, the coarse textured soils have been excluded from the group examined.

Atterberg recognized several consistency forms. He recognized the plastic consistency by his ability to roll the soil out into a wire. The moistness at which this just ceases to be possible he named the "Ausrollgrenze," or lower limit of plasticity. He considered the upper limit of plastic consistency, or the "Fließgrenze," to be that moisture content at which the bottom parts of a V-shaped furrow in a cake of soil, in a small, round-bottomed dish, just flow together when the dish is jarred. Commenting upon the "Fließgrenze," Atterberg stated (2, p. 37), "This is not a sharp, natural limit. Such a limit probably exists for loams but not for clays. Therefore the position of the limit must be considered as arbitrary." He made no statement of the number or magnitude of the impacts to be used, beyond stating that "the dish must be rapped violently and repeatedly against the palm of the hand" (ibid. 2, p. 37).

From a consideration of Bingham's fundamental conception of the flow of plastic solids having a high degree of homogeneity, when subjected to a shearing force F ,

$$Vk = \mu(F-f)$$

where V = volume of flow, k is a proportionality factor, μ is the coefficient of mobility² in analogy to the fluidity of liquids, and f represents the force necessary to overcome the internal friction of the material, or that just needed to start the flow. Figure 2 is a flow-shear diagram, after Bingham, for plastic solids possessing different degrees of mobility (as indicated by the slopes of the curves) and of internal friction, or yield values (as indicated by the intercepts on the abscissa).

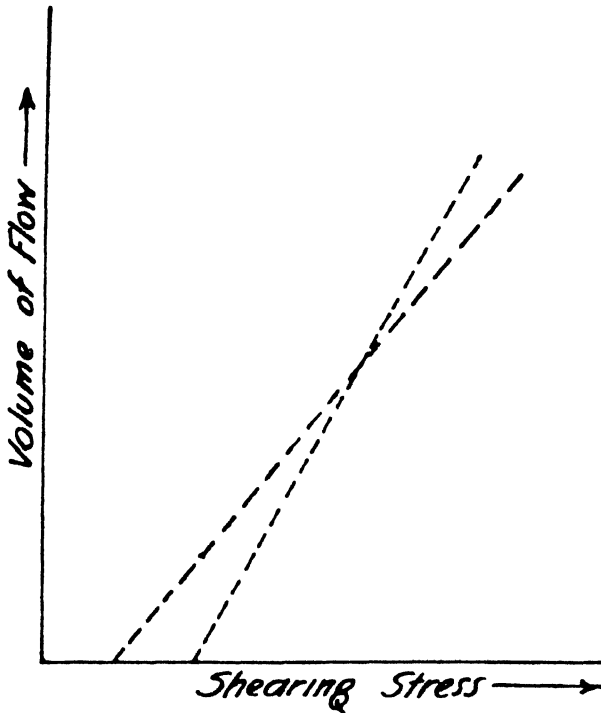


FIG. 2. FLOW-SHEAR DIAGRAM OF PLASTIC SOLIDS
(After Bingham)

By applying this idea to the case of a moist soil at the "Fließgrenze" of Atterberg, we may write

$$\mu (F - f) = 0$$

which is only true when $F = f$. As F approaches f as a limit the volume of flow approaches zero. It is therefore clear that insofar as Atterberg's conditions of measurement of the "Fließgrenze" can be reproduced, it must determine the moisture content of the soil at which the shearing force applied just exceeds the internal friction of the material and so causes a very slight flow to take

² Bingham defines the mobility of a plastic solid as the reciprocal of its consistency, where consistency is to be thought of as the measure of resistance to rapid deformation.

place. The so-called upper plastic limit must therefore be considered as entirely arbitrary, and dependent for its value upon the strength of the blow struck. Upon increasing either the impact force, or the number of impacts, provided each one always develops a shearing force slightly in excess of the yield value, the upper plastic limit will be lowered to a new moisture content such that the force necessary to overcome the internal friction of the soil is again just below the shearing force transmitted by the blow. It is conceivable that the "Fließgrenze" may be lowered at least to the "Ausrollgrenze" by increasing the shearing force. Russel and Wehr (15, p. 358) reported instances of practical coincidence of the two values, when determined in the ordinary way for certain soils. It follows that the plasticity number, or plastic range is also entirely arbitrary.

At the "Ausrollgrenze" the soil wire is subjected to a small shearing force when under a pressure slightly in excess of one atmosphere and must be in such a condition of moistness that shearing cannot occur unattended by crumbling.

Considering two different soils, at the same or different moisture content, which have in some way been subjected to the same shearing stress and which in consequence have produced equal amounts of flow,

$$V_1 k = \mu_1 (F_1 - f_1)$$

and

$$V_2 k = \mu_2 (F_2 - f_2)$$

In this particular case $V_1 = V_2 (= V$, the volume of flow) and $F_1 = F_2 (= F$, the shearing stress), μ_1 = the mobility of soil 1, μ_2 = the mobility of soil 2, f_1 = the internal friction or yield value of soil 1, f_2 = that for soil 2, and so,

$$\mu_1 (F - f_1) = \mu_2 (F - f_2)$$

and

$$\frac{\mu_1}{\mu_2} = \frac{F - f_2}{F - f_1}$$

Under these conditions, if $f_1 = f_2$, μ_1 and μ_2 will be equal. Different soils at such moisture contents that the application of equal shearing forces produces equal amounts of flow may be said to be of equal stiffness. That is,

$$\frac{\mu_1 f_1 - \mu_2 f_2}{\mu_1 - \mu_2} = F = \text{a constant,}$$

Otherwise stated, at the point of equal stiffness the flow-shear curves of different soils will intersect. It will be seen that this becomes possible for a wide range of soils, provided that their moisture contents may be adjusted. It may be

pictured more readily if a third axis, at right angles to the other two, is added to the coördinate system of figure 2, as in figure 3, to represent moisture content, provided that the system is always viewed in a direction parallel to the third axis. The consistency and yield value will vary with the moisture content.

By controlling the applied force, as for example in an adaptation of Atterberg's method by fixing the number and magnitude of the impacts, and by fixing the volume of flow, a rather simple means may be devised whereby the moisture content can be determined at which soils possess the same degree of

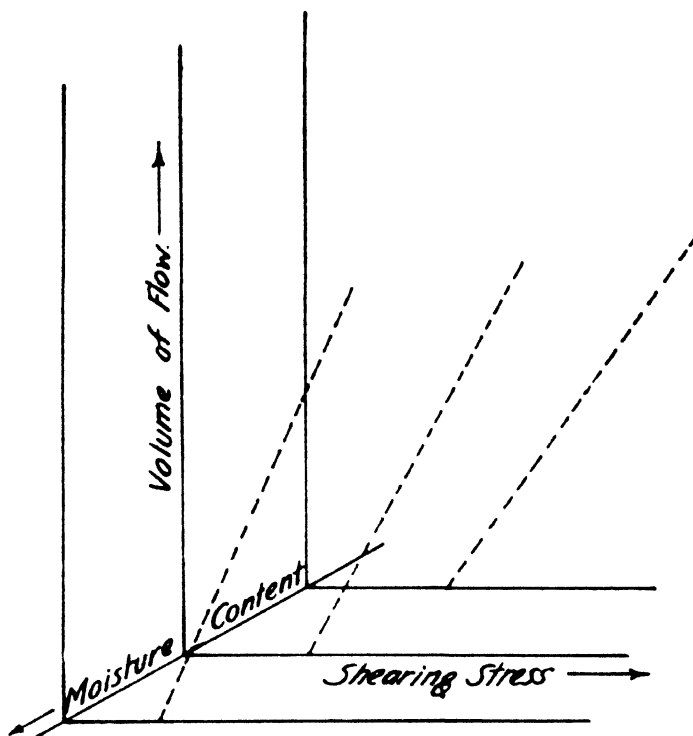


FIG. 3. FLOW-SHEAR DIAGRAM OF A PLASTIC SOLID, THE CONSISTENCY OF WHICH IS MODIFIED BY ITS MOISTURE CONTENT

stiffness. This was attempted in the present work, and the soils were examined as to moisture content when a certain small, fixed amount of flow was obtained under such conditions. It was first necessary to adopt some method for standardization of the impact magnitude. The physical basis for the method used is described in the following.

Dropping an object from a height to a fixed, rigid surface provides a simple way of subjecting it to a relatively large force during the very short time interval of impact. An expression for the magnitude of the force developed in this way may be calculated by consideration of certain elementary mechanical

principles. The momentum of a body falling freely from rest is given, for any instant, by the product of its mass m , and its velocity v ,

$$\text{momentum} = mv$$

which may be expressed³ in terms of force F , and time t ,

$$\text{momentum} = Ft$$

Upon striking the rigid surface at the distance a from its starting point the falling body almost immediately comes to rest, then⁴

$$\text{linear impulse} = \text{change in momentum} = -mv$$

The change in momentum is due to an impressed force F' , the magnitude of which at the time of impact may be measured by the change in momentum, and is given by

$$F' = \frac{m(v_2 - v_1)}{t'}$$

where v_1 = the initial velocity, v_2 = the final velocity, and t' = the time during which the force acts. In this case

$$F' = -\frac{mv}{t'}$$

Since $v = \sqrt{2gh}$, for unit mass $F' = -\frac{\sqrt{2gh}}{t'}$. The change in momentum, $-mv$, is brought about in the very small time interval t' , so that F' may be of considerable magnitude.

³ In the case of a freely falling body, neglecting air resistance, the acceleration, α

$$= \frac{d^2s}{dt^2} = g, \text{ the gravity constant}$$

$$\frac{ds}{dt} = gt + v_0 = v, \text{ the velocity at any instant}$$

and

$$s = 1/2 gt^2 + v_0 t + s_0 = \text{the distance fallen at any instant}$$

For a body starting from rest, where distance is measured from the starting point, V_0 and $s_0 = 0$, so that $s = 1/2 gt^2$ and $v = gt$. If $s = h$, the height, then $h = 1/2 gt^2$, and $v = \sqrt{2gh}$.

Since $F = m\alpha$, the mass may be expressed as $\frac{F}{g}$ and therefore momentum = $\frac{F}{g} \cdot gt = Ft$.

⁴ The linear impulse is given by measurement of the total change in linear momentum, which is obtained by measurement of the change in velocity.

If v_1 = velocity of falling body just before striking the rigid surface, and

v_2 = its velocity just after the blow has been struck,

Since $v_1 = v$ and $v_2 = 0$ cm./sec., then the

$$\text{impulse} = \text{change in momentum} = m(v_2 - v_1) = -mv.$$

EXPERIMENTAL

The procedure which involves these principles and which was finally adopted, follows. A moisture determination is made of the air-dry soil. Sufficient soil to give 20 gm. of water-free material is then weighed out into a small, round, straight-walled and flat-bottomed aluminum dish (50 mm. diameter x 13 mm. high). From the weight of dish and soil a table is previously prepared showing the gross weights corresponding to different moisture percentages over the working range. Water is then added to the soil from a burette and thoroughly mixed into it by manipulation with a stiff, short-bladed spatula. It is usually convenient to perform the initial mixing operation by repeatedly chopping the moistened soil with the edge of a spatula on a glazed tile. The moist soil is pressed in the puddled³ condition into the aluminum dish, smoothed level at the top, and weighed. It is then quickly provided with a V-shaped ditch having sides sloping at an angle of 45° with the perpendicular. The excavated wedge of soil is removed to a porcelain dish and placed in a humidifier during the dropping process which follows, for return to the soil mass after the ditch has been filled. The ditch should be as clean as possible, smooth-walled, with a right-angled vertex slightly above the bottom of the soil layer, of uniform width and depth each time and situated at the diameter of the dish. The impact used in the production of plastic flow is then provided by letting the dish fall freely and squarely from a height of 30 cm. to a heavy laboratory table. A given amount of flow is considered to have taken place when a succession of equal impacts has been provided, sufficient to fill the ditch. The number of impacts necessary is recorded. The exact moisture content as percentage on the dry basis may be calculated readily from the prepared table of gross weights by interpolation. By repeated additions and incorporations of water, weighings, ditchings, and droppings, data are obtained for the construction of a moisture content—impact curve. The maximum size of a ditch having the sectional form of an isosceles right triangle, vertex down, and in turn the amount of flow, are of course controlled by the depth of soil in the dish.

A ditch having a width at the top of 14 mm. and a depth of 7 mm. proved satisfactory. This gave a volume of about 2.3 cc. In all cases the size of the ditch was kept as nearly constant as possible. The width was controlled by parallel rulings on the smoothed surface with a caliper-like device cut from tin, the actual ditching being done with two razor blades held at an angle of 45° with the soil surface, and a spatula to remove the soil wedge.

Errors will be produced by non-uniformity in moisture distribution, incomplete puddling, construction of an imperfect ditch, high evaporation losses, and erroneous decision as to when the ditch is filled. The method of initial wetting has already been described. Later additions of water are usually more easily incorporated with the already moist cake by running the water on from a

³ For the present purpose a puddled condition is considered to be one in which the pore space of the soil has been sufficiently reduced that true plastic deformation may take place.

burette, cutting the soil checkerboard fashion with the spatula, and then thoroughly working up the soil. In order that none of the impacts shall be used up in puddling the soil care must be taken to prepare a well-puddled mass in the dish before ditching.

The preparation of a clean ditch of constant size is difficult with the stickier soils at high moisture contents. The loss of water during the dropping was found to be of relatively slight effect except in the drier stages. This was checked by making a moisture determination at the end of the run for each soil. The final water content was found to agree closely with that desired. With care, soil losses due to spattering are slight and seldom exceed 0.1 gm. in 20 gm. of soil.

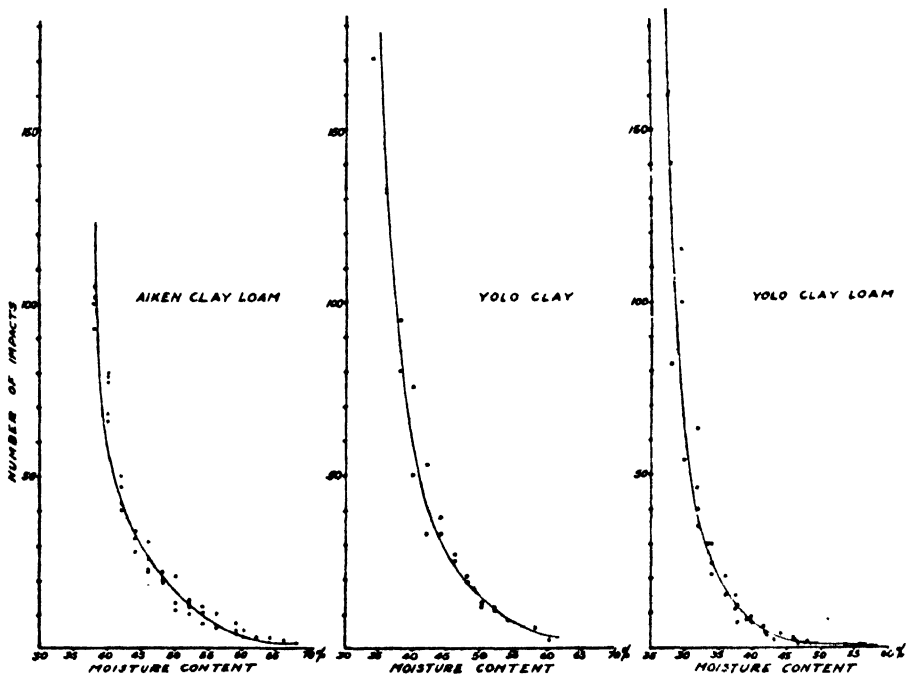


FIG. 4. MOISTURE CONTENT—IMPACT CURVES FOR AIKEN CLAY LOAM, YOLO CLAY, AND YOLO CLAY LOAM

Decision as to when the ditch may be considered filled presents some difficulty. The limit of readjustment of the soil mass after the construction of the ditch and consequent upon the dropping is clearly not reached until the surface of the soil cake is again level, but in a lower position than it originally occupied by an amount corresponding to the volume of the soil wedge excavated. In order to reduce evaporation losses, the ditch was considered to be filled when a number of blows had been delivered sufficient to cause the flattened walls of the ditch to be in practically continuous line (in cross section) with the very slightly sloping surface of the cake on either side of the ditch. This may be

judged by holding a straight edge at right angles to the line of the ditch, and resting vertically upon the soil.

No attempt was made to measure the absolute value of F' , a large part of the total force being consumed in various ways other than in the production of plastic flow. The important thing is that for unit mass, or any small element of the total soil mass, F' is reproducible with considerable accuracy, because of its direct proportionality to the square root of the height from which the soil falls, a value which is easily held constant.

When the number of impacts needed to produce a given quantity of flow are plotted against the corresponding soil moisture contents, curves of the power type are obtained. These are plotted for the Aiken clay loam, the Yolo clay, and the Yolo clay loam in figure 4. Several sets of values were obtained for each of these soils. An idea of the possible duplication of the results may be obtained from the position of the experimentally determined points with respect to the smooth curve drawn through them.

The relationship between the number of impacts needed to produce an arbitrary, fixed amount of flow in a certain soil and the water content of the soil, may be examined by assuming that $i = aw^n$, when $\log i = \log a + n \log w$, where i = the number of impacts needed to fill the ditch, w = total percentage of water (dry basis), and a and n are constants. By carefully plotting the experimental values of i and w upon coordinate paper to a logarithmic scale, a straight line is obtained with negative slope. Values for a and n are obtained by graphic solution, when a turns out to be large and positive, and n very much smaller and negative. The significance of the relationship becomes clearer if positive values of n are dealt with, in which case $i = \frac{a}{w^{-(n)}}$. This is the equation for a hyperbolic curve to which both coordinate axes are asymptotes. It will be seen that for a given soil, that is to say for fixed values of a and n , i increases in inverse proportion to some power of w . The curves Yolo clay loam and Yolo clay (fig. 4) are plotted to a logarithmic scale in figure 5. Their equations are

$$\begin{aligned}\text{Yolo clay,} \quad i &= (7.1 \times 10^{12}) w^{-4.92} \\ \text{Yolo clay loam, } i &= (1.3 \times 10^{12}) w^{-7.68}\end{aligned}$$

Impact number—moisture content measurements were made for 50 other soils. The results were plotted to a logarithmic scale and the straight line relationship was found to hold in every case. Figure 5 gives a few of these.

Moisture contents at which they have the same stiffness may readily be determined from a diagram which includes logarithmic graphs of the impact moisture relationships for all of the soils. Such a diagram was prepared, and the moisture contents compared when 100 impacts produced an equal amount of flow in all soils (table 2).

The other determinations reported in table 2 were carried out as follows:

1. *Air-dry moisture content.* About 5 gm. of soil was heated at 105°C. for 24 hours in glass weighing bottles, the loss in weight being reported as percentage of dry weight of soil.

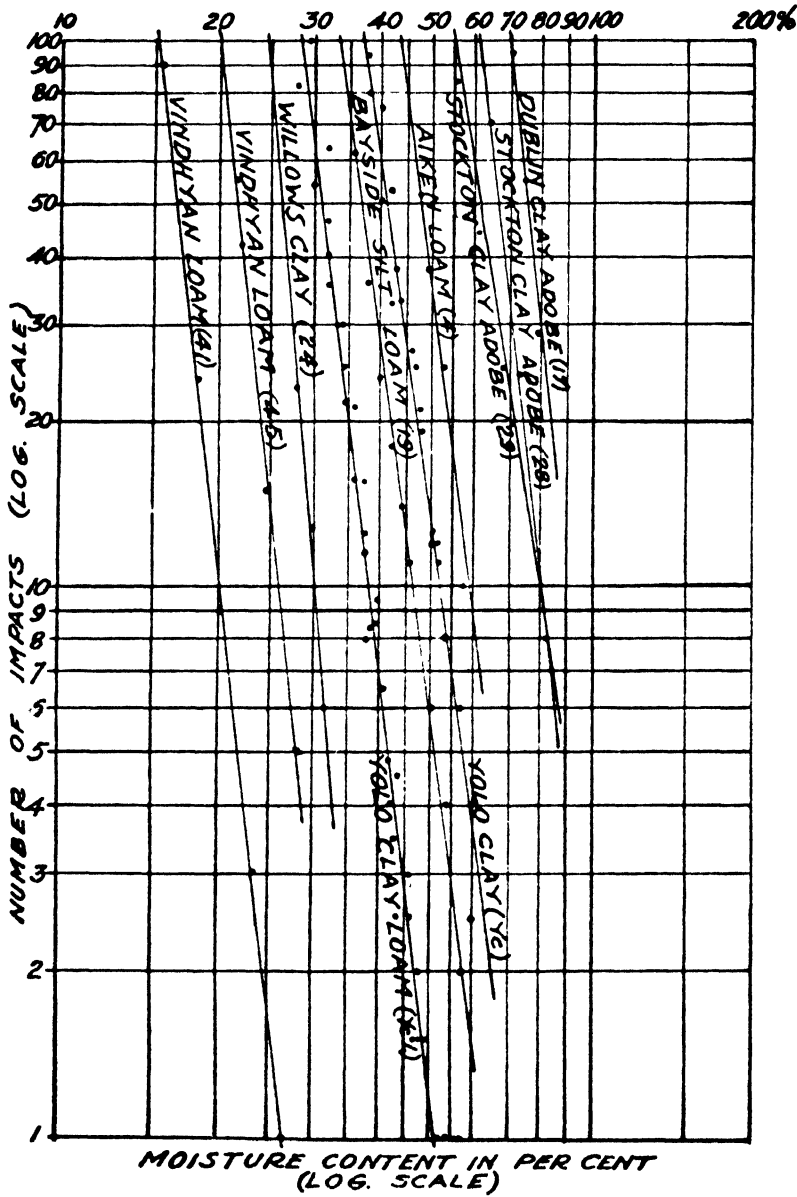


FIG. 5. MOISTURE CONTENT—IMPACT NUMBER CURVES PLOTTED TO A LOGARITHMIC SCALE

2. *Moisture equivalent.* Thirty grams of air-dry soil were used. The samples in the centrifuge cups were kept saturated in pans of water for 24 hours, drained for 30 minutes, and then placed in the standard centrifuge machine. The machine was brought up to speed

TABLE 2
Soil properties
 (Soils are arranged in ascending order of "water content at 100 impacts")

SOIL NUMBER	WATER CONTENT AT 100 IMPACTS	AIR-DRY MOISTURE CONTENT	MOISTURE EQUIVA- LENT	WATER VAPOR ABSORBED OVER 3.3 PER CENT H ₂ SO ₄	COLLOIDAL CLAY CONTENT	LOSS ON DIGESTION WITH HYDROGEN PEROXIDE	pH
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>gm. per gm. soil</i>	<i>per cent</i>	<i>per cent</i>	
41	15.2	1.02	16.5	.0413	13.8	1.13	6.05
45	19.8	1.85	18.6	.0652	21.7	0.85	6.36
44	21.2	2.02	20.2	.0650	21.7	1.67	6.37
42	21.6	2.07	20.2	.0556	18.5	1.55	5.75
24	24.6	2.31	20.9	.0595	19.8	1.99	6.44
33	27.2	4.74	27.0	.1012	33.4	1.28	8.48
43	29.0	2.57	20.1	.0721	24.0	1.18	7.54
6	29.2	6.18	23.1	.0862	28.7	0.45	6.39
10	29.3	4.67	26.3	.0925	30.8	2.38	6.70
40	31.4	4.02	23.7	.0815	27.2	2.44	6.43
25	32.2	3.57	32.2	.0981	32.7	1.22	6.65
49	33.4	4.31	27.0	.1052	35.1	0.47	7.76
19	33.4	2.75	27.3	.0642	21.4	2.96	5.77
18	35.6	3.73	28.2	.0957	31.9	3.33	6.60
22	36.0	6.42	33.2	.1258	41.9	2.97	7.78
11	37.0	6.05	28.1	.1228	40.9	2.13	6.87
50	39.4	5.47	27.3	.1105	36.8	0.89	7.78
12	43.0	6.82	29.8	.1367	45.6	1.68	6.62
5	43.0	5.39	29.1	.1163	38.8	1.51	8.09
4	43.2	4.80	35.3	.1701	56.7	1.53	5.55
36	43.4	4.22	29.6	.1215	40.5	0.69	7.97
47	44.6	6.92	35.2	.1487	49.6	0.53	7.97
38	46.0	4.53	29.2	.1445	48.2	0.40	8.14
48	46.0	5.99	34.8	.1481	49.4	2.09	7.59
3	46.0	7.74	33.7	.1502	50.1	3.36	6.62
27	47.0	5.52	28.9	.1651	55.0	1.15	6.22
20	47.5	3.12	39.1	.1104	36.8	4.81	5.62
37	47.5	4.50	33.3	.2031	67.7	0.30	7.99
16	49.2	5.59	32.6	.1309	43.6	4.11	6.85
8	49.5	6.04	37.7	.1527	50.2	2.74	7.02
26	49.5	5.99	33.4	.1358	45.3	2.50	7.08
2	51.0	7.54	44.3	.2623	87.4	1.57	7.51
1	51.5	7.16	33.7	.1590	53.0	1.57	7.94
39	52.0	4.95	32.8	.1275	42.5	1.14	8.48
14	52.5	7.73	35.5	.1686	56.2	2.46	6.74
21	53.0	5.56	38.6	.1764	58.8	2.43	9.13
32	54.0	6.86	33.0	.1464	48.8	1.09	6.19
34	55.0	9.31	35.4	.1670	55.7	0.84	8.65
29	55.0	6.53	34.8	.1500	50.0	1.26	5.85
30	55.5	6.68	43.0	.1516	50.5	1.03	6.37
31	55.5	6.82	30.6	.1492	49.7	1.92	5.66
13	56.4	7.82	43.1	.1697	56.6	3.17	6.71
15	58.0	6.13	43.4	.1523	50.8	4.67	6.30
35	58.6	9.91	37.6	.2037	67.9	0.68	8.71
9	61.0	8.13	38.1	.1629	54.3	1.57	7.16
28	61.0	7.90	42.8	.1575	52.5	0.81	7.18
23	63.0	8.96	39.4	.1879	62.6	1.97	6.94
46	64.0	8.47	41.5	.1952	65.1	1.38	8.11
17	70.0	9.67	49.3	.2000	66.7	2.99	6.95
7	71.0	11.5	43.2	.1140	38.0	5.43	7.21

gradually and maintained at between 2,450 and 2,475 r.p.m. for 30 minutes, after which the power was shut off and it was allowed to come to rest without the use of brakes. The soils were then rapidly transferred to covered cans and moisture determinations made in the usual way.

3. *Water vapor adsorbed over 3.3 per cent sulfuric acid.* The method of W. O. Robinson (14) was followed, with the exception that the soils were not ground to pass a 100-mesh sieve, previous experiments having shown this to be unnecessary. The adsorption was continued in partially evacuated glass desiccators, held at 30°C. for 5 days in a large air thermostat.

4. *"Colloidal clay" content.* An approximation to this value was obtained by dividing the water vapor adsorbed per gram of soil by 0.3 and multiplying by 100. In connection with some vapor pressure studies under way in this division the validity of the average factor 0.3⁶ as a means of calculating the approximate amount of colloidal material in soils was later examined for samples of colloidal clay separated from some other Californian soils. The results are given in table 3. The colloidal matter for the 5 soils was obtained by centrifuging at 35,000 r.p.m. from an aqueous suspension of clay having a maximum particle diameter of 0.001 mm., previously fractionated by sedimentation. No dispersing agent other than water

TABLE 3
Water vapor adsorption by different soil colloids over 3.3 per cent sulfuric acid for 5 days

SOIL NUMBER	NAME OF SOIL	WATER VAPOR ADSORBED PER GM. OF COLLOID
		gm.
321	Aiken (Horizon A)	0.283
322	San Joaquin (Horizon B)	0.298
323	Stockton subsoil	0.307
324	Yolo (Horizon A)	0.317
325	Peat	0.296
Mean.....		0.300

was used and the soils were not triturated. After removal from the centrifuge bowl the colloid was air-dried and gradually brought to constant weight over sulfuric acid. It was then ground to pass through a 0.25-mm. mesh screen and the powdered material used for the adsorption trials. No attempt was made to separate all of the colloid from the soils. There is close agreement in the average water vapor adsorption per gram of colloid with that obtained by Robinson. A narrower range was obtained, but that was to be expected, as only one-seventh as many soil colloids were used.

5. *Loss on digestion with hydrogen peroxide.* Two grams of oven-dried soil were weighed into a freshly dried 50-cc. Erlenmeyer flask, to the neck of which had been fused a 7-cm. length of glass tubing to act as a reflux condenser, and a shorter, slanting, side-tube to permit insertion of the soil. The flask and contents were next counterpoised against a similar, but empty, flask. Then 5 cc. of water and 10 cc. of 30 per cent hydrogen peroxide were added, the mixture was shaken, allowed to digest for 1 hour without external heating, placed on the steam-bath for 1 to 1½ hours, removed to an oven with the corresponding counterpoise flask, brought to approximate dryness, and then dried for an additional 24 hours, before being cooled and weighed against the same counterpoise cooled in the same way. The loss in weight, after correction was made for the solid residue remaining from the heating and evaporation of 10 cc. of the hydrogen peroxide, was considered as organic matter.

⁶ W. O. Robinson obtained a mean value of 0.298 gm. per gm. of soil colloidal matter.

6. *Hydrogen-ion concentration.* This was determined electrometrically with a Leeds and Northrup Laboratory Type Potentiometer, using a hydrogen electrode and a soil-water mixture in the ratio 1:2.

DISCUSSION

From the relationship $i = aw^n$, which is established in the present work for the higher values of w , it is evident that as the total water content diminishes the number of impacts necessary to produce the prescribed amount of flow increases indefinitely. This relationship is very similar to that obtained by Haines (9) when comparing the extrusion pressure in tons per square inch with the moisture content of certain porcelain clays. Haines experienced difficulty in the case of soils at the lower moisture contents because of interlocking of soil

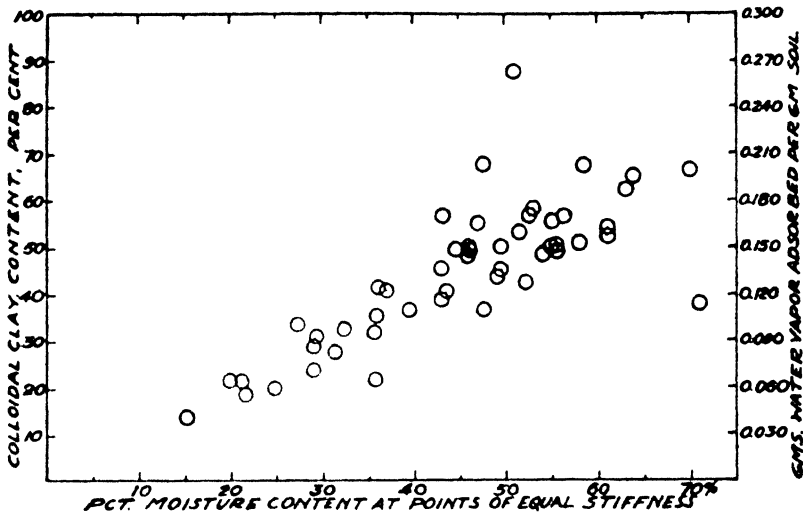


FIG. 6. RELATION BETWEEN COLLOIDAL CLAY CONTENT AND MOISTURE CONTENT AT POINTS OF EQUAL STIFFNESS

Colloidal clay content calculated from the amount of water vapor absorbed over 3.3 per cent sulfuric acid.

particles and squeezing out of water. It is doubtful whether the impact method could be used to examine the relationship for soils in the very dry condition because of the necessity of producing and maintaining a puddled condition, and of applying an impact force in excess of the yield value. Both methods present difficulties when it is desired to explore regions of low moisture content. However, it does appear that fairly heavy soils may be considered as plastics over a wider moisture range than is ordinarily conceded. The increase in plasticity with pressure, for a given moisture content, is well known among ceramic workers (12) and is explained by Haines (8) on the basis of a gradient in pressure of attraction between the clay particle and its water film on passing from the outer to the inner film layers.

The dependence of the moisture content at which one soil is of the same stiffness as another upon the actively water-vapor adsorptive material in the soil, is well indicated by the scatter diagram of figure 6. There is found to be a positive correlation of 0.797 between these two sets of values. According to the theory of plastic flow postulated by Searle (16) for clays, their plasticity depends upon the presence of a lubricating colloidal suspension surrounding the non-plastic particles. The present evidence indicates that the greater the quantity of the colloid in a soil the less perfect the lubrication between the aggregates for a given moisture content, and hence the less mobile the material. Increase in water content is accompanied by a thickening of the water films about the colloidal aggregates and a more ready shearing of the plastic mass. It appears reasonable to suppose that a change is brought about in both the yield value and the mobility, the former being decreased and the latter increased. At high moisture contents, where f is small as compared with the force produced by a single impact, and where the number of impacts and volume of flow are fixed, the soils evidently have very nearly the same mobility. Comparison of the moisture contents at which they have the same stiffness would then become one at which their mobilities were practically the same. The absolute measurement of the colloid content of a soil, if that is possible, and even the definition of the term "colloidal clay," are at the present time admittedly imperfect, and vague, yet from the observations recorded here it seems certain that the moisture content at which the stiffness reaches a certain value depends, for each soil, upon the amount present of a material which has as one of its properties a high adsorptive capacity, a typically colloidal characteristic. No attempt was made in the present work to investigate the nature of the colloidal material, other than by means of the hydrogen electrode, nor of its absorptive capacity for replaceable cations. According to Baver's observations (3) both of these properties may be expected to exert appreciable influence upon soils in the plastic state. Their effect upon the present group of soils, or upon other similar ones, is reserved for later investigation.

The scatter diagrams (figs. 7 and 8) obtained by plotting the air-dry moisture content and the moisture equivalent (table 2, columns 3 and 4) respectively, against the moisture content of the soils at points of equal stiffness (table 2, column 2) indicate the same close relationship ($r_{2,3} = +0.855$ and $r_{2,4} = +0.901$) to exist in these cases as is true for the correlation already given between the amount of water-vapor adsorbed and the moisture content of the soils at points of equal stiffness. The significance of the three coefficients may be examined most readily by reference to a table by Fisher (7, p. 176) in which are given values of the correlation coefficient for different levels of significance when different numbers of observations are made. From this table it is found that the probability that such correlations as have been obtained would arise by random sampling of an uncorrelated population is exceedingly low; namely, much less than .01 in each case. It is evident that a close relationship would be found to exist between these values at any of the comparable points upon

the logarithmic curves, because of the similarity in slope of the latter. The results of the experiment suggest that a scale of relative stiffness could be planned in which stiffness is measured by the number of standard impacts

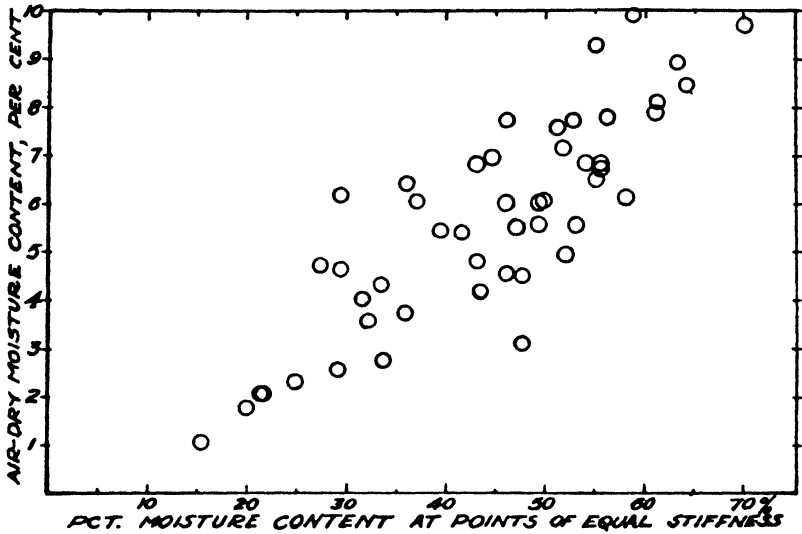


FIG. 7. RELATION BETWEEN THE AIR-DRY MOISTURE CONTENT AND MOISTURE CONTENT AT POINTS OF EQUAL STIFFNESS

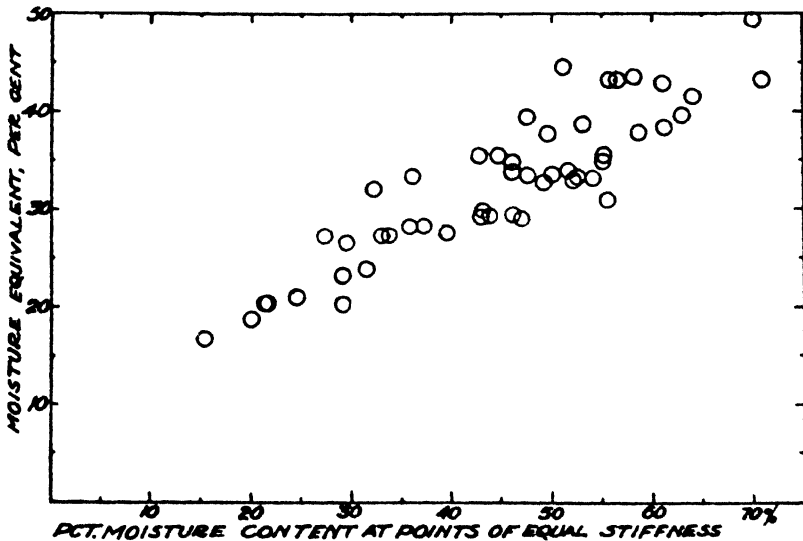


FIG. 8. RELATION BETWEEN THE MOISTURE EQUIVALENT AND MOISTURE CONTENT AT POINTS OF EQUAL STIFFNESS

needed to fill a ditch of standard size. In the present case, for example, the relative stiffness of the soils whose impact moisture curves are drawn in figure 5, may be obtained for any desired moisture content by a vertical section through the graph. It must be borne in mind, however, that extrapolation of the curves into regions of the lower values of w is not permissible beyond a point where the effective force of the impact is only just equal to the internal friction of the soil.

No relation is apparent between the order of arrangement of the soils at moistnesses of equal stiffness and their hydrogen peroxide decomposable organic matter. It must be concluded that for the soils examined, which are of rather low organic matter content, the latter simply behaves as an additional amount of colloidal material. Side-effects may exist for higher contents of organic matter. No distinct correlation was apparent between soil reaction and stiffness. The importance of the amount of colloidal material is thus further emphasized.

SUMMARY

A number of soils of different origin, family, stage of development, group, and series, and developed under different climatic conditions, were examined for several physical and a few chemical properties with regard to the relation existing between these properties and the behavior of the soils in the plastic state.

The soils used had moisture equivalents ranging from 16 to 51 per cent, colloidal clay contents, as measured by the water vapor adsorption method, from 15 to 64 per cent, pH values from 5.55 to 9.13, and were predominantly low in organic matter as measured by digestion with 30 per cent hydrogen peroxide.

By closer attention to, and greater control of, the details of the method originally devised by Atterberg for measuring the moisture content of soils at the so-called "Fließgrenze," and by consideration of the laws of flow of plastic solids developed by Bingham, a simple method was devised whereby the moisture content of soils may be compared at states of equal stiffness. Equal stiffness is defined as that consistency at which the application of an equal force produces in different soils an equal amount of flow. The method is described, and involves dropping a flat-bottomed, straight-sided, metal container, holding the plastic mass of soil of known moisture content, from a fixed height to a fixed, flat surface, until a ditch, previously excavated in the soil, is filled. By determining the number of impacts needed to fill the ditch at different moisture contents of the soil, and by graphically plotting the results, hyperbolic curves were obtained in all cases. There was found to be a close positive correlation between the air-dry moisture content, the moisture equivalent, and the colloidal clay content, respectively, and the soil moisture content at states of equal stiffness. This proved true for all degrees of stiffness, and

indicates that large quantities of water are bound by the soil colloidal material and that lubrication of the plastic mass is delayed when much colloidal matter is present.

The effect of natural soil organic matter, among the soils used, appeared no different from that of inorganic colloidal material. The hydrogen-ion concentration of the soils was without any distinct effect upon the amount of moisture needed to produce a given stiffness.

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SOME CHEMICAL PHASES OF SUBMERGED SOIL CONDITIONS

W. O. ROBINSON¹

U. S. Department of Agriculture

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In this paper the results of some investigations on the chemistry of submerged soils and submerged soil solutions are presented with the hope of throwing some light on the failure of such soils to grow satisfactory crops. Hitherto this failure has popularly been ascribed to lack of aeration, lack of nitrites, and other conditions caused by the lack of oxygen.

Preliminary laboratory experiments showed several specific causes of the toxicity of submerged soil solutions. These solutions differ markedly from aerated soil solutions in that they contain relatively large quantities of manganese and iron. Hydrogen sulfide and other poisonous gases are developed, and in cases where the soil is long submerged, the soluble bases are largely exhausted.

In the course of the investigation on the composition of submerged soil solutions, it was found that considerable gas was given off by submerged soils. This phase of the problem was studied, as it is closely allied to the composition of the solution and of the residual soils.

PRELIMINARY WORK

A corn field on Congaree silt loam near Colvin Run, Virginia, contained a poorly drained spot which was under water several times after the corn had reached a growth of about six inches. During most of the growing seasons there was little rain and the poorly drained spot was dry on the surface, but the corn did not grow on this spot. Samples of soil solution were taken in the middle of the poorly drained spot and also a few yards away, where corn grew exceptionally well. Qualitative examination showed an unusually large quantity of soluble manganese and iron and the water extracts of the soils were analyzed quantitatively for these elements. The results are given in table 1.

The data of table 1 show that the concentration of iron and manganese in the subsoil solution of the poorly drained spot is relatively very high. The subsoil solution of this spot is higher in manganese and iron than the surface soil solution and very much higher than the solution of the fertile adjoining spot. The water table of the poorly drained spot was about two feet below the surface and

¹ Soil chemist, Bureau of Chemistry and Soils.

the sample taken from the subsoil was submerged in water. There is abundant evidence in the literature that the concentrations for iron and manganese shown are sufficiently high to cause toxicity.

IRON AND MANGANESE IN BOG WATERS

The high iron and manganese content of the poorly drained spot of Congaree silt loam is a sufficient cause for its toxicity, and naturally leads to the examination of bog waters and poorly drained soil solution to see whether the observed condition is general. Accordingly, samples of solutions were taken from a few

TABLE 1

Comparison of iron and manganese in the water extract of well-drained and poorly drained spots of Congaree Silt Loam, Colvin Run, Va.

DEPTH OF SOIL	DRAINAGE	IRON AS Fe_2O_3	MANGANESE AS MnO
<i>inches</i>		<i>p. p. m.</i>	<i>p. p. m.</i>
0-7	Poor	1.4	2.0
40	Poor	17.0	28.0
0-7	Good	0.9	2.1
40	Good	2.1	2.2

TABLE 2

Iron and manganese in certain bog waters

LOCATION	Fe_2O_3	MnO
	<i>p. p. m.</i>	<i>p. p. m.</i>
Quarry in Falls Church, Va:		
Surface.....	1.6	1.0
18 inches deep.....	5.6	1.4
36 inches deep; in mud.....	8.7	4.2
Greenway Downs, Falls Church, Va.:		
About 48 inches deep; in mud.....	41.2	7.1
Vienna, Va.:		
About 4½ feet deep; in mud.....	2.9	4.3
Arlington Brick Yards, Va.:		
Surface.....	1.3	0.8
24 inches deep; in mud.....	2.1	1.1

nearby bogs in Virginia. There was considerable flow from two of the bogs, and samples were taken from the surface and also just beneath the mud at the bottom. The process of taking the solution was simple. The solution was filtered through a Pasteur-Chamberland tube. This tube was connected by a long rubber tube to the sample bottle which was exhausted by a hand pump. The first liter was discarded in order to get a representative sample.

The Vienna bog is an old natural one. The others are artificial and the bog near Falls Church is of recent origin. The results of the analysis are given in table 2.

Table 2 shows very variable quantities of iron and manganese. Both increase with depth where samples were taken at different depths. There is probably a surface dilution with rain and drainage water from better drained spots. The surface of the bog in Greenway Downs showed the peculiar iridescent film characteristic of chalybeate waters when they come in contact with air. Such waters contain ferrous carbonate and generally manganous carbonate held in solution by carbon dioxide. The ferrous iron at the surface is oxidized and precipitated as a thin film. The deep samples from the Arlington Brick Yards and from Vienna are not especially high in manganese and iron, presumably because these elements have been leached out. The deep sample from the bog near the Falls Church Quarry is higher in manganese and iron, as might be expected from the fact that the bog was of recent origin and probably contains considerable extractable manganese.

TABLE 3
Variation of solubility of manganese and iron with time of extraction
Kelley and McGeorge

SOIL NUMBER	DURATION OF EXTRACTION	MANGANESE AS MnO
		p.p.m.
313	1 hour	2 37
	24 hours	2.37
	7 days	14 1
314	1 hour	2.33
	24 hours	4 75
	7 days	11 9
319	1 hour	1.41
	24 hours	4 28
	7 days	7 07

It appears from these results that bog waters vary considerably in their content of manganese and iron. The quantities of these elements in river and well waters are interesting in this connection. Clarke (3) cites analyses of the Potomac, Mississippi, and James Rivers. The manganese content of these rivers varies from 0.05 to 0.22 p.p.m. of manganous oxide. The water from five wells in Falls Church, Virginia, contained from a trace to 0.15 p.p.m. of manganous oxide and from 0.24 to 1.3 p.p.m. of iron calculated as ferric oxide. Bog waters are therefore considerably higher in manganese and iron than are ordinary drainage waters.

IRON AND MANGANESE IN SUBMERGED SOIL SOLUTIONS

From the preceding data it is reasonable to expect that solutions of submerged soils will be high in iron and manganese. Kelley and McGeorge (18) have shown that the solubility of manganese in Hawaiian soils increases with time of contact with water. The soils they used were shaken with five parts of water in a stoppered bottle. Their results are given in table 3.

TABLE 4
Development of soluble iron and manganese with time of submergence

SOIL SERIES	DEPTH	TOTAL MnO IN SOIL	SOLUBLE MnO 1 DAY	SOLUBLE FeO ₂ 1 DAY	SOLUBLE MnO 8 DAYS	SOLUBLE FeO ₂ 8 DAYS	SOLUBLE MnO 22 DAYS	SOLUBLE FeO ₂ 22 DAYS	SOLUBLE MnO 60 DAYS	SOLUBLE FeO ₂ 60 DAYS	SOLUBLE MnO 80 DAYS
	inches	per cent	p.p.m.	p.p.m.	p.p.m.	p.p.m.	p.p.m.	p.p.m.	p.p.m.	p.p.m.	p.p.m.
Cahaba fine sandy loam, Georgia.....	0-12	.065	0.8	...	1.4
Cahaba fine sandy loam, Georgia.....	12-36	.024	None	...	None
Cahaba very fine sandy loam, Louisiana.....	0-12	.066	0.7	...	4.8
Cahaba very fine sandy loam, Louisiana.....	12-36	.079	Trace	...	0.1
Bermudian silt loam.....	0-8	.089	0.9	0.8	14.7	10.7
Carrington loam, Iowa.....	0-12	.057	0.5	0.4	1.9	4.3	4.4	20.0	34.0	40.0	...
Cecil clay loam, Georgia.....	0-9	.147	0.3	0.3	3.0	1.6	3.6	1.0	3.7	1.3	...
Chester loam, Virginia.....	0-7	.234	1.6	0.5	4.4	2.9	4.0	14.0	36.8	2.3	...
Chester loam, Maryland.....	0-8	.087	0.4	0.4	4.0	1.6	6.0	2.0	28.3	8.7	...
Clarksville silt loam, Tennessee.....	0-10	.372	1.2	Trace	11.4	1.0	21.6	4.0	44.4	2.8	...
Greenville sandy loam, Georgia.....	0-10	.030	0.4	...	0.8
Greenville sandy loam, Georgia.....	10-36	.016	0.2	...	0.3
Hagerstown loam, Maryland.....	0-8	.108	1.6	Trace	1.0	1.2	4.0	7.0	12.0	5.3	...
Huntington loam, Maryland.....	0-8	.263	3.7	1.1	24.2	15.6	36.4	50.0	50.0	33.0	...
Louisa loam, Virginia.....	0-7	.186	Trace	...	1.8	6.4
Manor loam, Virginia.....	0-7	.142	2.9	...	26.0	25.2
Manor loam, Maryland.....	0-7	.112	1.7	...	9.6	5.0	22.8	6.0	14.3	5.8	...
Memphis silt loam, Mississippi.....	0-6	.027	0.3	...	1.2
Memphis silt loam, Mississippi.....	6-36	.067	None	...	None
Memphis silt loam, smooth phase, Mississippi.....	0-6	.036	0.2	...	1.3
Memphis silt loam, smooth phase, Mississippi.....	6-12	.052	None	...	Trace
Norfolk sand, South Carolina.....	0-8	.028	0.4	...	2.1
Norfolk sand, South Carolina.....	8-36	.005	0.2	...	1.9
Norfolk fine sandy loam, Georgia.....	0-10	.020	Trace	...	1.1
Norfolk fine sandy loam, Georgia.....	10-36	.007	Trace	...	Trace

Norfolk fine sandy loam, Georgia	0-16	007	Trace	...	0.9
Norfolk fine sandy loam, Georgia	16-36	003	Trace	...	None
Orangeburg fine sandy loam, Georgia	0-10	.185	0.4	...	2.4	1.0	4.4	12.0
Orangeburg fine sandy loam, Mississippi	10-36	.595	Trace	0.6	0.5	0.6	0.6	1.0	0.5	0.8
Orangeburg sand, Georgia	0-10	.016	0.3	...	1.4
Orangeburg sand, Georgia	10-36	.011	0.3	...	1.2
Orangeburg sandy loam, Georgia	0-12	.054	0.4	...	1.6
Orangeburg sandy loam, Georgia Penn loam, Virginia	12-36	.027	0.2	...	0.7
		.188	2.0	...	13.8	33.6
Penn silt loam, Virginia		.042	1.2	...	9.7	64.4
Ruston fine sandy loam, Louisiana	0-6	.017	--	...	0.8
Sassafras loam, District of Columbia	0-7	.043	1.0	...	10.1	12.5
Tifton fine sandy loam, Georgia	0-12	.026	0.3	...	1.0
Tifton fine sandy loam, Georgia	12-36	.006	Trace	...	0.3
Wabash silt loam, Nebraska		.052	--	1.0	2.0	1.6	3.3	6.0	7.0	2.1

Table 3 shows a steady increase in the quantity of manganese dissolved with time of contact and a comparatively large content of manganese. The soils studied by Kelley and McGeorge were very high in alumina, iron, and manganese, differing considerably in this respect from American soils.

Experiments somewhat similar to those of Kelley and McGeorge have been carried out with American soils. For this purpose about 500 gm. of soil was placed in a bottle with about 2,500 cc. of water and the bottle stoppered. The bottles were shaken thoroughly once a day and samples of the solution were drawn at certain intervals, care being taken to admit as little air as possible. The samples were analyzed for iron and manganese. The results are given in table 4. The third column gives the total manganese in the soil and the remaining columns give the manganese and iron content of the solution in parts per million.

Table 4 shows that the solubility of manganese increases with time of submergence in all cases where there was any appreciable quantity of manganese in the soil. In general, the increase in the solubility of manganese with time of submergence, is greatest when there is an abundance of organic matter in the soil. Subsoils show very little initial solubility of manganese and the solubility does not increase much with time. Whether this is because of the form in which the manganese occurs or of the lack of suitable organic matter, is not known. In general, the subsoils are low in manganese, but one subsoil, that of the Orangeburg fine sandy loam, contained the largest quantity of manganese of any of the samples. The organic matter in this subsoil was but 0.77 per cent. The quantity of manganese made soluble is not proportional to the total quantity present, though, in general, soils low in total manganese show a low solubility. An exception to this generality is the Penn silt loam with only 0.043 per cent manganous oxide, which yielded a solution containing 64.4 p.p.m. In this soil three quarters of the manganese was made soluble. The maximum solubility for manganese was probably not reached in these experiments. The sample of Manor loam from Virginia which showed a solubility of 25.2 p.p.m. of manganese oxide after 80 days, yielded 60 p.p.m. after standing 10 years in a stoppered bottle.

The solubility of the iron also increases with time, but passes through a maximum with some soils. This may be due to the oxidation of ferrous bicarbonate and consequent precipitation of ferric hydroxide by the admission of a small quantity of air when the samples were taken. This oxidation takes place rather rapidly, for the submerged soil solutions generally showed the characteristic film of chalybeate waters after standing in the laboratory a few hours. It is possible that the precipitation of iron may be caused by the development of ammonia which, according to Subramanyan (32, 33), sometimes forms under submerged soil conditions. The sample of Manor loam from Virginia which had been standing in a stoppered bottle for 10 years contained only 1 p.p.m. of iron calculated as ferric oxide. There was a heavy precipitate of iron oxide in the solution just above the surface of the submerged soil. The Huntington

loam, Chester loam from Virginia, Hagerstown loam, Wabash silt loam, and Clarkesville silt loam show a decrease in the soluble iron from the 22- to the 60-day period. Iron is evidently precipitated more readily from chalybeate solutions than is manganese. This is in agreement with the observations of Fresenius (3, p. 532).

GENERAL COMPOSITION OF SUBMERGED SOIL SOLUTIONS

In the previous experiments it was noted that some of the submerged soil solutions contained comparatively large quantities of calcium and magnesium. The determination of these elements in submerged soil solutions has little bearing on the toxicity of submerged soils. If, however, the solubility of calcium, magnesium, and other elements is greatly increased by submerged soil conditions, these elements may be dissolved to such an extent as to render the soils unproductive. Accordingly a number of the submerged solutions were analyzed for silica, alumina, iron, manganese, lime, magnesia, potash, and soda. The corresponding analysis of the fresh solutions were also made. The analyses are given in table 5.

Table 5 shows a great increase in the solubility of lime and mangesia as well as an increase in iron and manganese, which has been noted before. Silica and presumably alumina remain much the same as in the beginning, except that the solubility of the silica is somewhat increased in the soils containing much organic matter. Soils low in organic matter such as the Cecil and Orangeburg show very little actual increase in any of the constituents.

Potash and soda show a considerable actual increase in all cases. There is undoubtedly some soil mineral decomposition going on during the long time of submergence. The great increase of the lime, magnesia, iron, and manganese, due mainly to the presence of carbon dioxide, probably causes base exchange processes to be operative. The concentrations of potash and soda would be increased by this cause.

According to the figures given, there is but little increase in the solubility of alumina with time of submergence. Stoklasa (31) reports that a specimen of marsh water contained 273 p.p.m. of alumina. This specimen must have been very acid, for Magistad (22) found that a pH of 3.96 was required to hold 200 parts of alumina in solution. The pH of the solutions examined was not less than 5.0, which is capable of holding only about 2 p.p.m. of alumina in solution. The presence of other soluble material tends to lower rather than to raise the solubility of the alumina, according to Joffe and McLean (15). It is apparent, therefore, that the solubility of alumina is not increased by submerged soil conditions.

The solubility of lime is so high under submerged soil conditions that such a soil would soon lose all the lime it contained, provided, of course, that there was movement of the solution or movement of the lime by diffusion. From a comparison of the composition of the solutions given in table 5 with the composition of the soils given in a previous bulletin (26) it can be seen that soils

TABLE 5
Increase in solubility of various elements with time of submergence

SOILS AND LOCATION	DEPTH	ORGANIC MATTER	IN CONTACT WITH SOLUTION 18 HOURS										SUBMERGED 60 DAYS																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																
			SiO ₂	R ₂ O ₃	MnO	CaO	MgO	K ₂ O	Na ₂ O	SiO ₂	Al ₂ O ₃	Fe ₂ O ₃	MnO	CaO	MgO	K ₂ O	Na ₂ O																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																												
			p.p.m.	p.p.m.	p.p.m.	p.p.m.	p.p.m.	p.p.m.	p.p.m.	p.p.m.	p.p.m.	p.p.m.	p.p.m.	p.p.m.	p.p.m.	p.p.m.	p.p.m.	p.p.m.																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																											
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long submerged would contain little but silica and alumina. This depletion of essential elements is given by Hilgard as a prominent cause of the unproductiveness of poorly drained soils.

EFFECT OF CARBON DIOXIDE ON THE SOLUBILITY OF SUBMERGED SOIL CONSTITUENTS

It will be shown later that gases formed from submerged soils contain carbon dioxide. Solutions saturated with this gas have the property of dissolving large quantities of lime and magnesia, as is well known. If a flask partially filled with some of the more concentrated submerged soil solutions is evacuated, precipitation of manganese, iron, calcium, and magnesium takes place. Those elements are evidently held in solution by carbon dioxide. It is self-evident that the solubility of lime and magnesia in soils would be increased

TABLE 6
Influence of carbon dioxide on the solubility of manganese

SOILS AND LOCATION	MnO DISSOLVED IN 24 HOURS	
	Distilled H ₂ O	CO ₂ -saturated H ₂ O
	p.p.m.	p.p.m.
Bermudian silt loam, Virginia	0.9	15.0
Louisa loam, Virginia	0.1	13.9
Manor loam, Virginia	2.9	28.9
Penn loam, Virginia	2.0	23.8
Penn silt loam, Virginia	1.2	3.3
Sassafras loam, District of Columbia	1.0	3.6

by bubbling carbon dioxide through the soil. Presumably the solubility of iron and manganese would be likewise increased. Some experiments were carried out in this connection. A slow stream of carbon dioxide was bubbled through a one to five mixture of soil and water for 24 hours. Manganese was determined in these solutions and also in solutions of the same soils in the absence of carbon dioxide. The results are given in table 6.

Table 6 shows that the solubility of manganese is greatly increased by the presence of carbon dioxide. Several factors are operative here. The reaction is made more acid, calcium and magnesium go into solution as bicarbonates, and a greater exchange of bases takes place. The solution being saturated with carbon dioxide, any manganous or ferrous compounds formed would be dissolved by the carbon dioxide. The solubility of iron is presumably increased by the presence of carbon dioxide, for there is every reason to believe it would act like manganese in this respect.

INFLUENCE OF AIR VOIDS ON THE DEVELOPMENT OF SOLUBLE IRON AND MANGANESE

In the experiments on the development of soluble iron and manganese, (table 4) some air was let into the bottles when samples of the solution were taken. It seemed possible that the oxygen of the air admitted would have some effect on the solubility of the iron and manganese, though the volume of air was comparatively small. To test this point, three soils were submerged in such a manner that nitrogen instead of air replaced the withdrawn solution. Samples were withdrawn at an interval of 30 days. The iron and manganese contents of the resulting solutions are given in table 7.

It is apparent that the introduction of such quantities of air are not significant but it is not to be concluded that soil solutions are independent of the presence or absence of air. When solutions from submerged soils are allowed to stand in an open beaker they become cloudy, and a reddish brown precipitate soon forms. The same precipitate is formed when air is slowly bubbled

TABLE 7

Comparison of iron and manganese solubilities in stoppered bottles with air and nitrogen voids

SOIL AND LOCATION	30 DAYS DURATION, 1 5			
	Nitrogen-filled void's		Air-filled voids	
	Fe ₂ O ₃	MnO	Fe ₂ O ₃	MnO
	p. p. m.	p. p. m.	p. p. m.	p. p. m.
Carrington loam, Iowa.....	34	8 0	31	9.6
Chester loam, Virginia.....	18	11 6	16	10.2
Hagerstown loam, Maryland.....	14	6.6	12	7.6

through the solution. The iron is first deposited. Long continued contact with air produces a separation of a portion of the manganese.

THE REACTION OF SUBMERGED SOIL SOLUTIONS

The reaction of submerged soil solutions is of interest in connection with the toxicity of such solutions. It is also important as a possible cause of the greatly increased solubility of the bases, particularly of ferrous iron and manganese. The solubility of the last two named elements is greatly increased with increasing hydrogen-ion concentrations of soil solutions.

It is commonly supposed that submerged soil solutions are more acid than aerated solutions of the same soil. On the contrary, Gillespie (8) and Subramanyan (32) have found that submerged soil solutions are less acid than aerated solutions. Subramanyan found that the nitrogen compounds in the rice paddy fields rapidly develop ammonia, which decreases the hydrogen-ion concentrations.

The hydrogen-ion concentration of a number of soils and solutions resulting from 60 days submergence has been measured. In all cases, as shown in table 9, the gas in contact with the solutions contained carbon dioxide. The apparatus used to measure the hydrogen-ion concentrations was of the bubbling cell type and is not particularly well adapted for this purpose, since the dissolved carbon dioxide is rapidly displaced by the hydrogen. The readings were therefore taken as quickly as possible and another reading was taken after the hydrogen had been bubbled through the solution for 60 minutes. The results are given in table 8.

Table 8 shows that the reading when first taken was usually about pH 6. The readings were very variable and rose rapidly at first. The Cecil and Orangeburg series behaved differently from the others. There was very little

TABLE 8
Reaction of certain soils and submerged soil solutions

SOILS AND LOCALITY	SOIL	SOLUTION	
		First read- ing	After 60 minutes bubbling
	pH	pH	pH
Carrington loam, Iowa	5.18	7.2	7.60
Cecil clay loam, Georgia	5.44	6.0	6.22
Chester loam, Virginia	6.33	6.5	8.10
Chester loam, Maryland	6.50	6.6	8.00
Clarksville silt loam, Tennessee	6.20	5.5	7.93
Hagerstown loam, Maryland	7.58	6.2	8.53
Huntington loam, Maryland	5.83	6.6	8.36
Manor loam, Maryland	5.80	6.0	7.93
Orangeburg fine sandy loam, surface, Mississippi	5.12	4.5	4.48
Orangeburg fine sandy loam, subsoil, Mississippi	4.70	4.7	4.70
Wabash silt loam, Nebraska	..	6.0	7.50

change in the readings with time in these two soils and the Orangeburg surface changed to a lower rather than a higher reading with time. Table 8 shows that the Cecil and Orangeburg soils developed very little soluble constituents with submergence, a property doubtless connected with this behavior.

After hydrogen had been passed through the solution for about an hour the majority of the readings became constant (for any one soil) at a pH value varying between 7.5 and 8.5. This point is probably governed by the reaction of the carbonates precipitated from the solution by the displacement of the dissolved carbon dioxide by hydrogen.

The foregoing values were taken after the solution had been submerged 60 days. Since Subramanyan (32, 33) has shown that submerged soils give off ammonia at some stages of submergence, it is possible that these solutions might have been alkaline at some earlier stage. It would be difficult to recon-

cile this with the observed continually increasing concentration of iron and manganese in solution. It would seem that the pH would be governed by the carbon dioxide evolved and this was sufficient to nearly saturate the solution each time when tested.

GASES FROM SUBMERGED SOIL

In nearly all the experiments it was noticed that gas was given off by the submerged soil mass. The solutions are therefore saturated with these gases and their composition may be expected to have an influence upon the toxicity of the solutions. A study of the composition of these gases was therefore undertaken.

The gases from submerged soils may be expected to resemble the gas sometimes arising from marshes. This "marsh gas" consists mainly of methane with a small quantity of carbon dioxide, and occasionally hydrogen is reported.

Gases from submerged soils owe their origin to the decomposition of organic matter by anerobic bacteria. Omelianski (25) has identified a number of such soil bacteria. These microorganisms produce mainly methane and hydrogen and in some cases small quantities of hydrogen sulfide and mercaptans.

Harrison and Subramania Ayer (10) determined the composition of a large number of gas samples from rice paddy fields. They found that the gas dislodged by poking the soil in a submerged and recently transplanted rice field consisted largely of methane with small quantities of hydrogen, carbon dioxide, nitrogen, and oxygen. In the gases arising normally from the surface of a growing rice field, methane and hydrogen disappear and the gas consists of nitrogen, carbon dioxide, and oxygen. They account for the difference of composition of the gases developed by assuming that the blue-green algae growing on the paddy fields decompose the marsh gas and convert it into carbon dioxide and water and the hydrogen into water.

The gases were generated and collected in the following manner. Four hundred or five hundred grams of soil were placed in a 2½-liter bottle. The bottle was entirely filled with water, making about a one to five mixture. After this mixture had stood two or three days the fine bubbles and floating organic matter collecting on the top were floated off by water and the bottle was stoppered and connected to an inverted flask or endiometer entirely filled with water. In this way water displaced the air in the entire apparatus. The connection was so made that the arising gas displaced the water in the flask or endiometer. The end of the outlet tube was placed several inches under water so there was no opportunity for the air to suck back into the collecting chamber. Under these conditions gas commenced to appear after from two weeks to two months. The rates of gas evolution varied greatly with different soils and at different times with the same soil. In one case 1,000 gm. of soil yielded 250 cc. gas daily; with another soil about 5 cc. was given off in the same time. In general, the gas evolution was more rapid at higher than at lower

temperatures. In all cases gas was eventually produced. No attempt was made to effect microbiological control. The analyses of the gases obtained are given in table 9.

The analyses given in table 9 show the gases to be mainly methane, generally with some hydrogen, nitrogen, carbon dioxide, oxygen, and in some cases carbon monoxide. Qualitative examination showed the presence of a small quantity of sulphur in the gases.

The observations of Harrison and Subramania Ayer (10) are confirmed with respect to the disappearance of hydrogen and the formation of a large quantity of nitrogen in submerged soils where blue-green algae are present. It will be noted that no hydrogen was given off by the Carrington, Hagerstown, Cecil, and one sample of the Wabash soils. The solutions from these soils supported a

TABLE 9
Composition of gases from submerged soils

	METHANE	HYDROGEN	NITROGEN	CARBON DIOXIDE	CARBON MONOXIDE	OXYGEN	ILLUMINANTS
	per cent	per cent	per cent	per cent	per cent	per cent	per cent
Carrington loam*	66.0	None	20.1	8.6	None	3.7	None
Cecil clay loam*	54.5	None	35.8	6.8	None	2.7	None
Chester loam	67.5	7.8	14.7	4.7	None	3.2	2.1
Hagerstown loam*	58.2	None	30.1	7.5	..	4.2	None
Huntington loam†	64.0	15.9	..	1.8	2.8	..	0.8
Huntington loam	64.8	16.3	7.3	3.9	3.2	3.2	1.2
Huntington loam	66.8	24.2	1.9	3.1	3.0
Orangeburg fine sandy loam	65.7	12.5	11.8	3.7	None	3.8	2.5
Wabash silt loam†	47.0	25.0	..	3.7
Wabash silt loam*	16.2	None	62.8	15.2	None	5.8	None

* These mixtures supported a heavy growth of blue-green algae.

† There was some air contamination in these samples and consequently a complete analysis was not made.

heavy growth of blue-green algae. The methane of the gases from three of these four soils is lower than the gases from the other soils and the nitrogen is very much higher. The blue-green alga has been identified by N. R. Smith of this bureau as a member of the Chlamydomonos group. When blue-green algae appeared soon after the submergence of the soil the evolution of gas was invariably delayed and the evolution proceeded at a much slower rate than where no algae were present.

The occurrence of carbon monoxide appears to be unusual in marsh gas, and the analysis was checked in several ways. The percentage of carbon monoxide was determined by absorption by cuprous chloride. Under some conditions the alkaline pyrogallol used for the absorption of oxygen, previous to the carbon monoxide determination, may give off some carbon monoxide. However,

several analyses made on gases from other soils in the same manner showed only a trifling absorption, within the limits of experimental errors. The presence of carbon monoxide in the two different samples of gas from the Huntington loam was confirmed by tests with Hoolamite tubes (12). Tests with these tubes on a sample of gas from the Huntington loam showed the presence of a comparatively large quantity of carbon monoxide. Gases from the Wabash and Hagerstown soils, which showed a carbon monoxide absorption so trifling as to be well within the limits of experimental error, gave no test for carbon monoxide by the Hoolamite tubes. The presence of carbon monoxide in gases from submerged soils seems never to have been reported. Löhnis (21), Mulder (23), and Corenwinder (5) report the formation of small quantities of carbon monoxide from manure. Langdon (18) found that the gases of the floaters of the giant help contained from 1 to 12 per cent carbon monoxide.

The composition of the gases has an interesting relation to the composition of the solutions. In all cases carbon dioxide was present in the gas phase in considerable quantities and ferrous iron, manganese, calcium, magnesium, and perhaps other elements were held in solution as bicarbonates. The production of carbon dioxide simultaneously with the reduction of iron, and, probably of manganese, by the action of the anaerobic bacteria on the organic matter accounts for the observed leaching of iron from poorly drained places subjected to the "watery maceration" mentioned by Hilgard (12).

The fact that hydrogen is formed by the microorganisms shows that very strong reducing conditions prevail and free ferric oxide can not be expected to escape reduction under submerged soil conditions.

The quantity of hydrogen sulfide, mercaptans, and other sulfur-containing gases formed is, of course, limited by the quantity of sulfur in the soil. Although there is a very small quantity of sulfur in the evolved gases, or in the solution, there is considerable ferrous sulfide deposited on the sides of the containing bottle and throughout the soil mass. In the Huntington loam, more than three-quarters of the total sulfur had been converted to sulfides after 60 days submergence. This was shown by the quantity of hydrogen sulfide evolved on boiling the submerged soil with dilute hydrochloric acid. Murray and Irvine (24) found a similar precipitation of iron sulfide in the blue muck of the littoral sea bottom when the mud was mixed with decaying organic matter and allowed to stand under water. Rost (27) found sulfides of iron in the lower layers of certain Minnesota peats.

The quantity of organic matter consumed in the production of methane, carbon dioxide, and hydrogen presents an interesting phase of this work. From 100 gm. of Carrington loam 1,365 cc. of gas was produced in about three months. This quantity of gas contained 0.69 gm. methane and 0.2 gm. carbon dioxide. On the assumption that this material is derived solely from the organic matter of the soil it represents about 25 per cent of the organic matter present. A sample of the Huntington showed a more rapid decomposition. A kilo of this soil yielded 250 cc. gas daily. At this rate the organic matter

would be exhausted in about 60 and 70 days. Probably a larger quantity of organic matter went into the solution and into the bodies of the bacteria. Hence the disappearance of soil organic matter under submerged soil conditions may be very rapid, as Hilgard (12) suggests.

The destruction of soil organic matter under submerged soil conditions, as described in the foregoing, appears paradoxical in view of the accumulation of peat in large quantities in horizons submerged in water.

An explanation may be found in the creation of a toxic condition in the peat, as Jeffrey (14) has held. Waksman,² however, considers that bacteria do not function in peat because they are unable to subsist on the lignin which is the only carbohydrate left in peat, the cellulose and other carbohydrates having disappeared in the earlier stages of peat formation. The organic matter of the recently submerged soils would contain organic matter other than lignin and therefore support the growth of bacteria. I. C. Feustal of this bureau reports some experiments that are significant in this connection. He submerged three samples of a peat profile and measured the gases given off. After six months no gas had been given off by the samples from the deeper profiles, and the surface sample had given but a few cubic centimeters. The deeper samples were then inoculated with a very active submerged soil and in four months no gas has been given by those samples. Thiessen, Reinhardt, and Johnson (34) consider it proved that bacteria exist and function in peat at all depths. In view of Mr. Feustal's experiments, however, we are forced to conclude that some samples of peat do not contain gas-producing bacteria and furthermore are toxic to such bacteria. Jeffrey (14) holds that yellow spores of bacteria are present even in coals and appear as indestructible as the gold they resemble. He considers that peat in the deeper profile is antiseptic and bacteria are prevented from growth thereby. The toxicity, however, is not sufficient to kill the spores, which may develop under proper conditions. Jeffrey's viewpoint seems to clarify the situation. If spores are present and can germinate, cultures would be obtained when peat samples were put in the proper environment, but it would not follow that bacteria were functioning in the peat.

Gillespie (8) has shown that a strong reduction potential is set up in a few hours in soils submerged by a thin film of water in an open beaker. A disagreeable odor is developed as the reduction potential is set up. This odor is due to hydrogen sulfide or other hydro-sulides. In this laboratory it has been found that the foul odor develops generally with soils in a short time after an air-dried sample is submerged. These facts suggest two deductions: First, it is apparent that the anerobic bacteria develop rapidly in all soils when the proper conditions obtain; and, second, the sulfur in the soil is rapidly changed to sulfides. The strong reduction potential has a bearing on the toxicity of submerged soil solutions and will be discussed later.

² Privately communicated.

TOXICITY OF SUBMERGED SOILS

Livingston (20) showed that bog water usually contains some toxic substance. Dachnowski (6), following the procedure of Breazeale (1) on aerated soil solutions, showed that the toxicity of bog waters is decreased by being shaken with carbon black or calcium carbonate. He also showed that the toxicity of these waters is not due to acidity nor to lack of oxygen.

Experiments reported earlier in this work show that bog waters and submerged soil extracts are high in manganese and ferrous iron and that sulfides are present.

Manganese in the concentrations found is clearly toxic to some plants but not to others. Skinner and Reid (30) found that there was a beneficial effect of manganese on wheat seedlings in concentrations as high as 50 p.p.m. Brenchley (2) found 4 p.p.m. toxic to peas. Gössl (9) states that marsh and water plants contain more manganese than do land plants. Evidently marsh and water plants tolerate much more manganese than do land plants, and other examples could be cited to show that manganese is much more toxic to some land plants than to others.

With ferrous iron the case is very different. Ferrous iron appears to be toxic to land plants in general in concentrations even as low as 3 to 5 p.p.m. There is great difficulty in keeping the iron in a ferrous condition in culture solutions and it is probable that lower concentrations of ferrous iron are toxic. Ruprecht, (28) found that 4 p.p.m. of ferrous iron was toxic to clover seedlings, and Hartwell and Pember (11) showed that 3 p.p.m. was toxic to barley and rye. Skeen (29) found 1 to 2 p.p.m. to be toxic to lupines and Phaseolus. Practically all surface soils containing more than a very small quantity of organic matter develop sufficient ferrous iron to be toxic after being submerged a few days.

Sulfides are produced in all submerged soils. This can be shown by passing the gas over lead acetate paper, by the blackening of the soil mass, and by treatment of the blackened soil with hydrochloric acid with the evolution of hydrogen sulfide. The presence of sulfide-forming bacteria in soils is general, according to Waksman, (35).

Hydrogen sulfide in all concentrations is very poisonous to plants. The concentration in soil solution is never large, being regulated by an excess of iron and other bases in solution. On the other hand, the sulfide concentration is increased by the fact that the solubility of ferrous sulfide is increased by the acidity due to the carbon dioxide in solution. The solution of the Huntington loam after 60 days submergence contained about 14 p.p.m. of soluble sulfides.

Stoklasa (31) reports as high as 273 p.p.m. of alumina in marsh water. Our analyses show only 1 or 2 p.p.m., and the pH of the solutions is such that there can be little alumina in solution. The toxicity of submerged soil solutions therefore cannot be due in general to dissolved alumina.

Nitrites, which are also toxic, develop when well-aerated soils are submerged. Kelley (17) found that when nitrates were added to rice pot cultures and the cultures submerged, nitrites rapidly developed in toxic quantities.

The gaseous constituents present in small quantities such as illuminants, mercaptans, and occasionally carbon monoxide, although toxic, are present in such small quantities as probably to be of little importance.

The toxicity of submerged soils is therefore due to several constituents of which sulfides and ferrous iron are probably the most important. Toxic concentrations of these develop rapidly after the soil is submerged.

DISCUSSION OF RESULTS

The high manganese and iron content of bog waters and of submerged soil solutions has not been emphasized by earlier workers in discussions of the toxicity of such waters. The concentration of manganese observed in submerged soil solutions is not always sufficient to be toxic. With iron and probably hydrogen sulfide, toxic concentrations develop rapidly. It is quite possible that the toxic concentrations of iron, manganese, and hydrogen sulfide observed may be but an effect of a general reducing condition which, in itself, is the main and most quickly effective toxic agent.

Dachnowski (6) has stated that the toxicity of bog waters is not due to the lack of dissolved oxygen. When the surface of the water is open to the air, as in natural conditions, oxygen diffuses rather rapidly from the top downward, as Subramanyan (32) has shown. In such submerged soils, whether the reactions are oxidizing or reducing, will depend upon the rate at which oxygen is consumed by the microbiological activities compared to the rate of oxygen diffusion from the surface. In the experimental work reported here, however, there can be but very little dissolved oxygen after that originally present in the water has been consumed by microorganisms or displaced by the evolution of other gases. The reducing action in submerged soils protected from the air is so intense that hydrogen is commonly given off. The chemical reaction going on under these reducing conditions must be radically different from reactions taking place in aerated soil solutions. The reducing action of submerged soils must in itself be toxic, for it is not to be supposed that the plant can carry on the same normal root absorption in a reducing environment that it does under oxidizing conditions. The lack of dissolved oxygen in submerged soil solutions must be an important factor in studies of the toxicity of such soil solutions. It is beyond the scope of this paper to consider the action of reducing conditions, *per se*, on the plant. The discussion is therefore confined to such specific effects of this reducing condition as the increase in the concentration of certain inorganic constituents and the production of sulfides.

The experiments performed give us very little idea of the time necessary for soils to become toxic after submergence. In general, toxic concentrations for iron are developed within 8 days. Concentrations of manganese sufficient to be toxic are not always developed in this time. The soils used in the experimental work were air-dried. Since the microorganisms are more active in fresh soils in place, it is probable that soils submerged under field conditions would develop toxic concentrations of manganese and iron in considerably less

than 8 days. Soils submerged for 24 hours generally develop a foul odor, and hydrogen sulfide can be detected in the soil gases.

There is reason to believe that the subsoil solution may differ considerably from that of the surface soil particularly in the iron and manganese content. The subsoil is frequently submerged when the surface soil is not, and part of the surface soil solution has access to the air. Some data recorded have shown that subsoil solutions, taken at the lowest depths of the B horizon, are considerably higher in iron and manganese than the corresponding surface soil solutions.

It is probable that considerable solid matter is transferred by circulation and diffusion from a part of the soil which is submerged to another part having access to the air. Ordinarily this process would work in the direction of the depletion of the lower horizon and the enrichment of upper horizons having both an oxidizing reaction and contact with the solution of the submerged horizon. Mottled spots in poorly drained soils doubtless owe their origin to some such process as this.

Some time ago Comber (4) advanced the hypothesis that plants must be able to absorb colloidal iron from the soil, since the iron found in the soil solution was totally inadequate to account for the iron found in the plant. Plant roots extend into the subsoil and even below. When the water table is high it would be a very easy matter for the solubility of the iron to be so increased as to make considerably more iron available to the plant. The same submerged subsoil conditions which cause more soluble iron should also cause more soluble calcium, magnesium, and manganese.

Poor plant growth on soils that have been submerged but later adequately drained, may be due to lack of essential elements. Hilgard (12) has discussed the destructiveness of this "watery maceration," and the results given here have shown how much more soluble iron, manganese, calcium, and magnesium are produced under submerged soil conditions than under conditions of good drainage. The destruction of the organic matter or certain parts of it, at least, goes on with surprising rapidity.

In poorly drained spots where there is no loss of inorganic elements by slow leaching or diffusion, the loss of organic matter still goes on, and it is apparent that practically all the available organic matter would be consumed by the microorganisms at a rapid rate. Unless organic matter was being added to such a soil by plant growth, the lack of organic matter would be a serious problem if the soil were reclaimed by drainage.

The increase in the solubility of iron under submerged soil conditions has an interesting application in some problems of plant physiology. Gile and Carrero (7) found that rice plants suffering from lime-induced chlorosis when grown in a limestone soil, turned a normal green color when the soil was submerged for 10 days. The correct explanation of this observation was given by Johnson (16), who reasoned that the reducing conditions brought about by submerging the soil so increased the solubility of the iron as to overcome the chlorosis. Pineapples grown on Hawaiian soils high in manganese suffer from chloro-

sis due to lack of available iron. Liberal applications of stable manure to the high manganese soils of Hawaii prevent chlorosis of the pineapples, though this treatment is not so effective on lime-induced chlorosis in Porto Rico. Presumably the beneficial action of stable manure on the Hawaiian soil is due to the reduction of the insoluble ferric compounds to soluble ferrous compounds. Green manuring should also effectively bring about reducing conditions and is a possible clue to the cure of chlorosis under some conditions.

The facts brought out by this work have a possible bearing on the selection of materials for construction of water gardens. Surface soils high in organic matter and also high in manganese and iron will yield a solution high in iron and manganese. Whereas water plants are probably tolerant to higher concentrations of these elements than are land plants, there may be plantings which would be injured by too high concentrations. On the other hand, old leached water gardens may be rejuvenated by the addition of surface soils rich in organic matter, iron, manganese, and other elements that have been leached away. The addition of soils high in manganese and iron but having no organic matter will have little effect in increasing the soluble iron and manganese, since in the absence of organic matter there is no reducing action to increase the solubility of these elements.

The presence of carbon monoxide in the gases from some submerged soils has a bearing on the unhealthy conditions observed in some swamps. It is not claimed that marsh gas normally contains carbon monoxide but it is believed that under some conditions marsh gas may contain small quantities of carbon monoxide.

SUMMARY

1. Submerged soil solutions are radically different from aerated soil solutions in that they contain high concentrations of iron and manganese. The iron and manganese are present as proto bicarbonates. Submerged soil solutions are also high in calcium and magnesium and contain hydrogen sulfide and other sulfides.

2. The high concentration of iron, manganese, calcium, and magnesium is caused indirectly by the microbiological action on the organic matter, which produces carbon dioxide along with other gases. It is the carbon dioxide that is mainly responsible for holding the iron, manganese, calcium, and magnesium in solution.

3. In the absence of organic matter the solubility of iron, manganese, calcium, and magnesium is not increased under submerged soil conditions.

4. Soils are not made more acid by submergence for short periods except by such acidity as is due to carbon dioxide and bicarbonates.

5. All normal soils containing organic matter, when submerged, eventually produce gas. This gas production is retarded by blue-green algae of the *Chlamydomonas* group. When blue-green algae are absent, the gas consists mainly of methane and hydrogen. In the presence of the algae the hydrogen

and part of the methane are decomposed. Under these conditions the gas is mainly nitrogen and carbon dioxide with variable quantities of methane.

6. Soil organic matter in aerated soils disappears, in some instances very rapidly, when the soils are submerged.

7. Submerged soil solutions develop toxic concentrations of ferrous iron, sulfides, and commonly manganese. Toxic concentrations of these elements are occasionally developed in bog waters and in solutions of poorly drained soils, and they are invariably developed in submerged soils protected from the air. Soils that have been submerged for a long time may be so profoundly leached of calcium, manganese, manganese, and iron that they will not support plant growth.

8. Toxic concentrations of ferrous iron and sulfides develop in a few days after submergence. Toxic concentrations of manganese develop somewhat more slowly.

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SOME FERMENTATION CHARACTERISTICS OF VARIOUS STRAINS OF RHIZOBIUM MELILOTI AND RHIZOBIUM JAPONICUM

R. H. WALKER AND P. E. BROWN

Iowa State College

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The physiological characteristics of the legume bacteria have been studied by a number of investigators in recent years for the purpose of developing a laboratory method which would serve to differentiate the various cross-inoculation groups. Among the more promising physiological tests that have been used for this purpose are those involving the fermentations of carbohydrates and other carbon compounds.

Baldwin and Fred (1) reported that when these organisms were grown on agar media of low buffer capacity and containing brom-thymol-blue, their fermentation characters were sufficiently definite to permit of a separation into groups corresponding to the groups established by cross-inoculation tests. They report further, that in many of the cross-inoculation groups, subdivisions could be established by this method of differentiation. Such subdivisions were made in the alfalfa and clover groups.

Studies on the physiology of these bacteria by Walker (6) showed that there was a wide variation among organisms of the same cross-inoculation groups when they were compared on the basis of their ability to change the reaction of sugar media. It was also pointed out that the variations in this action were as great within a single cross-inoculation group as among the organisms of different groups. The results of the fermentation tests were such that it did not appear justifiable, however, to separate the strains of the organisms into subgroups within the cross-inoculation groups.

Schönberg (5) studied the behavior of 18 strains of legume bacteria, representing a number of the more common species, on nutrient carbohydrate agar containing brom-thymol-blue. These organisms were also studied in solution cultures of the same media without agar. The carbohydrates used were glucose, galactose, xylose, and sucrose. It was found that the solution cultures were better suited for differential diagnostic purposes because of the wider variations in reaction changes produced. Schönberg concluded that the differences in reaction changes were not constant enough to serve as a basis for characterizing and separating the legume nodule bacteria.

It should be noted that Schönberg studied only one strain of some species of this group of bacteria, which was the case for *Rhizobium meliloti*, and only a

few strains of other species. Two strains of *Rhizobium japonicum* were studied. Whether a true picture of the behavior of a group of organisms toward carbohydrate media can be obtained by studying only one or two strains of organisms of that group is very doubtful. In fact, the data presented by Schönberg indicate that there are significant differences in fermentative ability among strains of a single species.

Because of the apparently great variability in the fermentative powers of different strains of single species of the legume bacteria, and since this physiological characteristic has been used as a differential character in the separation of species (2), it was purposed to study this question in further detail in order to ascertain, if possible, the extent of the variations, and also to study the constancy of the fermentative power of individual strains of bacteria.

PRELIMINARY STUDIES

As a preliminary measure of the fermentative characters of these bacteria, a number of individual strains of organisms from the various cross-inoculations groups were grown on agar slants containing different indicators. A basic culture medium of the same composition as that employed by Baldwin and Fred (1) was used. It had the following composition:

Dibasic potassium phosphate	0.5 gm.
Sodium chloride	0.2 gm.
Magnesium sulfate	0.2 gm.
Calcium carbonate	3.0 gm.
Agar	15.0 gm.
Sugar	10.0 gm.
Yeast extract	100.0 cc.
Distilled water	900.0 cc.
Indicator, 0.5 per cent alcoholic sol.	5.0 cc.

The reaction of the medium was adjusted to pH 7.0. The indicators used were brom-thymol-blue, and brom-cresol-purple. Glucose, galactose, and mannose were used as sources of energy. The results of these tests were quite interesting, especially when the strains of *Rhizobium meliloti* (2) and *Rhizobium japonicum* were compared. The majority of the *meliloti* strains produced an acid reaction, which resulted in a yellowish color when brom-cresol-purple was used as indicator in the agar. On the other hand, the majority of the *japonicum* strains produced an alkaline reaction and changed the indicator to a deep red. In the first comparison of the groups as a whole, the strains of the two species appeared to be distinctly differentiated by this test, but after closer observation it was noted that not all of the *meliloti* strains produced an acid reaction in the medium. The indicator was not changed in some tubes, and was even a deeper red in other tubes than in the uninoculated control tubes. Likewise, not all of the *japonicum* strains produced an alkaline reaction in the medium, and in such cases the indicator remained unchanged in color or only slightly changed.

The same variations were noted when brom-thymol-blue was used in the medium, but were not so pronounced because of the different pH range of the indicator. The results with this indicator, however, did show marked variations in the fermentative abilities of different strains of single species of bacteria.

Because of the relatively narrow range of a single indicator and the impracticability of making exact pH measurements on agar slopes by comparison with standard indicator solutions, this method of testing the fermentation characters of these bacteria was inadequate and only qualitative in nature.

It was clearly evident from this preliminary work that in order to make a detailed study of the constancy of the fermentative power of a single strain of bacteria and to study the extent of the variability among strains of the same species of this physiological character, the work must be put on a quantitative basis. In order to do this the following method was adopted after a number of preliminary trials had been made.

METHODS USED

A culture medium of the same composition as that employed in the preliminary studies, except that no agar or indicator was added, was prepared and sterilized in flasks containing an amount sufficient for each separate test. Sugar was then added at the rate of 1 per cent of this sterile solution. The sugars used were glucose and galactose. After the sugar was completely dissolved the solution was pipetted into 50-cc. Erlenmeyer flasks, 10 cc. being placed in each flask. The flasks were plugged and sterilized in the autoclave at 15 pounds pressure for 15 minutes. The solutions were then inoculated with a loopful of bacteria taken from a young agar slant. The cultures were incubated at about 28°C. for 100 hours, after which time pH measurements were made by the quinhydrone electrometric method.

The change in hydrogen-ion concentration in the medium was taken as a measure of the fermentative ability of the strain of organisms in that medium. To serve as a control in these tests, two or three flasks of the uninoculated culture medium were incubated along with the inoculated cultures. The difference between the pH of the control cultures and the inoculated cultures was considered to represent the change in reaction produced by the organisms.

The small Erlenmeyer flasks were used instead of test tubes, as the organisms were found to produce greater reaction changes when grown in a shallow layer of solution having a comparatively large surface exposure.

For these studies 23 strains of *Rhizobium meliloti* and 12 strains of *Rhizobium japonicum* were used. The fermentation powers of these organisms were tested in the glucose and galactose media under similar conditions a number of times in order to determine the extent of the variations among strains and the constancy of each individual strain.

RESULTS

The 23 strains of *Rhizobium meliloti* were grown in the yeast-water-glucose medium in eight consecutive tests, and in the yeast-water-galactose medium

in five consecutive tests. The results of these tests are given in tables 1 and 2 respectively. The 12 strains of *Rhizobium japonicum* were likewise grown in the yeast-water-glucose medium in five consecutive tests and in the yeast-water-galactose medium in five tests. The results obtained in these tests are shown in tables 3 and 4.

TABLE 1

Changes in reaction produced by 23 strains of Rhizobium meliloti in eight consecutive tests in a yeast-water-glucose medium

Incubated 100 hours. Results expressed as pH

STRAIN	1	2	3	4	5	6	7	8
Control	6.82	6.55	6.87	6.75	6.76	6.79	6.79	6.79
1	5.84	6.54	6.04	6.00	5.90	6.38	5.97	6.30
2	7.55	7.24	7.77	7.65	7.44	7.25	7.28	7.27
3	6.10	6.29	6.34	6.32	6.28	6.20	6.23	6.25
4	5.85	6.09	6.04	6.17	5.95	6.68	6.69	6.78
5	6.00	6.22	6.21	6.29	6.14	6.25	6.21	6.28
6	5.77	6.12	6.02	6.05	5.89	5.97	5.90	6.03
7	6.52	6.70	6.75	6.79	6.43	6.61	6.49	6.51
8	6.13	6.27	6.31	6.19	6.11	6.31	6.33	6.30
9	5.77	6.20	6.09	6.07	5.93	6.17	6.08	6.18
10	6.23	6.30	6.36	6.32	6.30	6.28	6.21	6.32
11	5.74	6.30	5.78	5.57	5.61	6.13	6.25	6.11
12	5.99	6.06	5.99	6.01	5.95	6.05	6.01	6.00
13	5.84	6.17	6.00	6.05	6.03	6.17	6.00	6.00
14	5.64	6.01	5.71	5.66	5.61	5.87	5.68	5.77
15	5.85	6.16	5.38*	5.59*	5.94	6.05	6.08	6.17
16	6.70	6.67	6.89	6.83	6.80	6.87	6.85	6.82
17	6.78	6.86	7.04	6.88	6.92	6.93	6.93	6.93
18	5.94	6.61	6.02	5.98	5.91	6.25	6.28	6.18
19	5.94	6.22	5.92	5.78	5.99	6.20	6.25	6.17
20	6.58	6.89	6.63	6.49	6.60	6.83	6.87	6.83
21	6.10	6.26	6.32	6.27	6.15	6.32	6.72	6.37
22	6.00	6.30	6.34	6.39	6.06	6.78	6.37	6.47
23	7.57	7.18	7.78	7.57	7.63	7.22	7.27	7.28

* These cultures were probably contaminated.

Constancy of the fermentative powers of individual strains

It can be seen quite readily from these tables that the reaction changes in the consecutive tests with the same organisms on the same medium are very similar. This would seem to indicate that the fermentative powers of individual strains of bacteria are approximately the same in the various consecutive tests, and that the fermentative ability of a particular strain of organisms is a comparatively constant character.

Although the exact amount of change brought about by each strain of bacteria was not the same in all tests, it did bear about the same relationship to the

changes produced by the other strains of the same bacteria. The variations in the conditions under which the experiments were conducted undoubtedly are responsible in large measure for the variations in the exact amount of change produced by an individual strain of organisms in consecutive tests. For example, the reaction of the culture media before inoculation could not be brought to exactly the same pH in each test because of the inherent difficulties in the methods that must necessarily be employed. Variations in the steam pressure during sterilization, and in the length of the sterilization period have

TABLE 2

Changes in reaction produced by 23 strains of Rhizobium meliloti in five consecutive tests in a yeast-water-galactose medium

Incubated 100 hours. Results expressed as pH

STRAIN	1	2	3	4	5
Control	6.87	7.00	6.97	6.97	6.97
1	6.57	6.67	6.41	6.38	6.39
2	7.07	6.96	7.07	7.15	7.10
3	6.14	6.37	6.26	6.24	6.28
4	6.23	6.74	6.26	6.34	6.23
5	6.17	6.40	6.28	6.30	6.32
6	5.97	6.12	6.02	5.99	6.14
7	6.81	6.30	6.68	6.68	6.65
8	6.34	6.34	6.41	6.42	6.42
9	6.13	6.42	6.27	6.30	6.27
10	6.26	6.45	6.23	6.24	6.18
11	6.29	5.77	6.02	5.88	5.90
12	6.08	6.10	6.07	6.07	6.07
13	6.13	6.19	6.14	6.17	6.14
14	5.67	5.48	5.65	5.59	5.57
15	6.11	6.22	6.05	6.05	6.02
16	6.76	6.64	6.65	6.76	6.76
17	6.81	6.67	6.77	6.82	6.84
18	6.24	6.44	6.18	6.20	6.18
19	6.10	6.24	6.27	6.27	6.27
20	6.87	6.47	6.53	6.67	6.62
21	6.18	6.39	6.04	6.41	6.28
22	6.21	6.39	6.42	6.44	6.39
23	6.97	7.14	7.12	7.24	7.14

slight influences upon the final pH of medium. Only by standardization of the methods of procedure can these slight variations be reduced to a minimum. In this work, and especially in the later tests, an attempt was made to standardize the procedure to such an extent as to eliminate, as far as possible, all the variations due to this cause. In spite of these precautions, however, there remained some variations in the media at the time of inoculation.

Other factors responsible in large part for the variability of the reactions of an individual strain of organisms from time to time are the amount of inoculum

used and the stage of growth of the organisms at the time of inoculation. These factors each have a decided influence upon the rate of growth of the organisms, as has been pointed out by Henrici (4) and by Chesney (3). They

TABLE 3

Changes in reaction produced by 12 strains of Rhizobium japonicum in five consecutive tests in a yeast-water-glucose medium

Incubated 100 hours. Results expressed as pH

STRAIN	1	2	3	4	5
Control	6.82	6.55	6.87	6.75	6.76
1	7.60	7.39	7.77	7.77	7.71
2	6.85	7.25	7.09	6.79	6.87
3	7.45	7.58	7.77	7.56	7.79
4	7.55	7.50	7.71	7.54	7.64
5	7.58	7.22	7.22	7.49	7.75
6	7.67	7.32	7.75	7.46	7.66
7	7.71	7.41	7.78	7.62	7.66
8	7.62	7.44	7.78	7.44	7.77
9	7.35	7.30	7.39	7.26	7.33
10	7.63	7.32	7.77	7.60	7.74
11	7.65	7.09	7.78	7.62	7.75
12	7.62	7.36	7.78	7.64	7.73

TABLE 4

Changes in reaction produced by 12 strains of Rhizobium japonicum in five consecutive tests in a yeast-water-galactose medium

Incubated 100 hours. Results expressed as pH

STRAIN	1	2	3	4	5
Control	6.87	6.98	6.86	6.86	6.68
1	7.07	7.53	7.53	7.61	7.58
2	6.96	7.26	7.24	7.17	7.26
3	6.91	7.04	7.10	7.02	6.94
4	7.43	7.51	7.61	7.57	7.58
5	7.37	7.60	7.57	7.54	7.54
6	7.17	7.12	7.58	7.56	7.54
7	6.89	7.68	7.47	7.54	7.53
8	7.29	7.26	7.36	7.54	7.53
9	7.55	7.84	7.79	7.70	7.81
10	6.87	7.71	7.59	7.56	7.56
11	7.31	7.41	7.57	7.46	7.51
12	7.11	6.35	7.59	7.51	7.46

found that when a culture is transplanted during a period of maximum growth rate, it continues to grow at a maximum growth rate in the new medium. But if transplanted at a time when the organisms are in the resting period or in the period of no growth, they show a lag in the new medium. Henrici states

further, that the morphologic variations of bacteria are but outward manifestations of equally profound variations in the physiology of the organisms. He states that there are scattered references in the literature to differences in the fermentative power between young and old cultures.

It is obvious, then, that slight variations in the fermentative power of an individual strain of bacteria would be expected in consecutive tests unless such factors as the size of inoculum and the phase of growth at the time of inoculation, were controlled. And even if the size of inoculum were accurately controlled, not all of the cells would grow at the same rate, and there would be a selection of the most rapidly growing strain from the inoculum as has been shown by Henrici (4). It would seem, then, that these factors would influence considerably the extent of the fermentation in a limited incubation period.

It is also very obvious that these factors could not be absolutely controlled for routine work. It would be almost impossible to inoculate fermentation media in a number of consecutive tests with a large number of strains of bacteria, variable as they are in physiology, with the same number of organisms in the same phase of growth in each case. In the studies reported here, however, an attempt to standardize these conditions has been made, by inoculating with approximately the same size of inoculum, as measured by a loop, and by transferring from cultures of approximately the same age. The organisms of course were not necessarily in the same phase of growth.

In spite of the fact that all the conditions of the various tests could not be controlled and were not absolutely identical it seems very evident from the data that the fermentative characters of an individual strain of these bacteria are fairly constant.

The fact that individual strains of bacteria reacted similarly in consecutive tests, even though the environmental conditions were not absolutely identical, seems to substantiate further the conclusion drawn in the foregoing and give additional weight to it. It seems very probable that if all environmental conditions, such as reaction and composition of the medium, temperature and time of incubation, size of inoculum and stage of growth of the bacteria used, could be controlled in such a way that all tests were conducted under absolutely identical conditions, the results obtained in the fermentation of a sugar by a particular strain of bacteria would be very nearly the same in consecutive tests.

Variations in the fermentative powers of different strains

These data also indicate quite clearly that there are large variations in the fermentation characters of different strains of the same species of bacteria. This is shown by the results of the tests in glucose medium with practically all the cultures, but especially so with cultures 1, 2, 11, 14, 16, 17, and 23 of *Rhizobium meliloti* and with cultures, 1, 2, 9, and 12 of *Rhizobium japonicum*. In every case, cultures 2 and 23 of the *meliloti* species produced a very alkaline reaction in the glucose medium; 16 and 17 changed the reaction but slightly,

making it more alkaline in all but one case; 1, 11, and 14 produced a strongly acid reaction in the medium. In the third and fifth tests on the glucose medium, strain 14 produced a reaction over 100 times as acid as that produced by strain 23. Other strains produced reactions intermediate between these extremes. In the case of the *japonicum* strains, 2 changed the reaction but

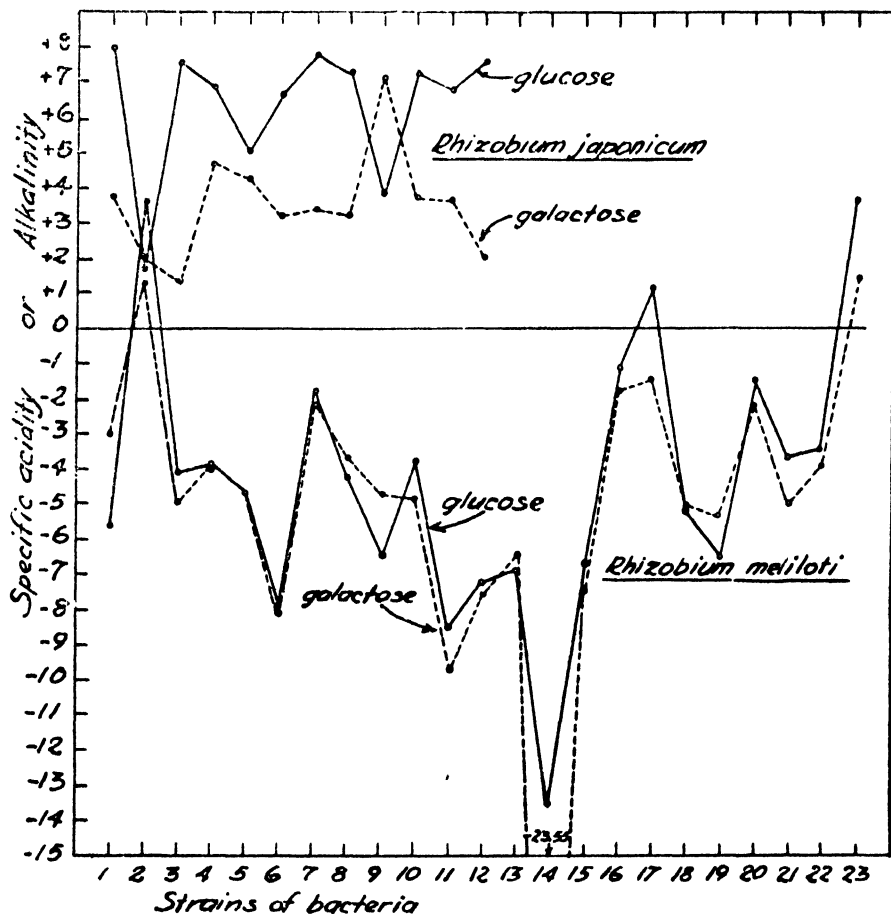


FIG. 1. VARIATIONS IN AMOUNTS OF SPECIFIC ACIDITY OR SPECIFIC ALKALINITY PRODUCED BY 23 STRAINS OF *Rhizobium meliloti* AND BY 12 STRAINS OF *Rhizobium japonicum* WHEN GROWN IN YEAST-WATER GLUCOSE AND YEAST WATER-GALACTOSE MEDIA

slightly in the glucose medium; 1 and 12 produced a rather strongly alkaline reaction, and 9 produced an intermediate reaction. Some of the strains of this species produced reactions almost ten times as alkaline as those produced by strain 2.

The variations between strains in ability to alter the reaction of media is also shown when the data for the fermentation of galactose are compared.

Strains 2 and 23 of the *meliloti* species made the medium more alkaline in all but one case, whereas strain 14 brought about a very strongly acid reaction of the medium. Strains 16 and 17 produced only very slight changes in the reaction of this medium, making it slightly more acid than the control. Other strains varied in their fermentative powers between these two extremes.

In the case of strain 3 of the *japonicum* species a very slight change in the galactose medium was produced. Strain 9, however, produced a fairly alkaline reaction in this medium. The other strains varied between these extremes in reaction effects.

The extent of the variations in the fermentative powers of different strains of these two species of *Rhizobium* in glucose and galactose media is further illustrated in figure 1. In this figure, graphs have been made of the averages of the actual amounts of acidity or alkalinity produced in the media. Because of the fact that when results are expressed in terms of pH, as they are in the tables already referred to, the figures are reciprocals of the logarithms of the numbers. When data of this type are plotted the differences between strains appear to be less variable than they really are. For this reason, the actual amounts of acidity or alkalinity produced have been plotted in terms of specific acidity or specific alkalinity as suggested by Wherry and Adams (7), in order that a true comparison of the fermentative abilities of the different strains of bacteria may be made.

These graphs show very clearly the high degree of variation between strains of the same species in the ability to ferment glucose and galactose.

Effects of growth upon different sugars

Another interesting relationship brought out by these data and shown in the graphs, is the fact that the various strains of *Rhizobium meliloti* ferment glucose and galactose with about the same comparative results in the changes in reaction. On the other hand, the strains of *Rhizobium japonicum* do not appear to ferment these two sugars in the same manner.

Comparison of the fermentative powers of different species

One very important fact shown by these data is that although the reactions produced by the majority of the *japonicum* strains are much more alkaline than those produced by the majority of the *meliloti* strains, this was not true in all cases. For example, the reactions produced by the *meliloti* strains 2 and 23 were as alkaline as some of the *japonicum* strains in both of the sugar media. *Meliloti* strain 17 produced a reaction in the glucose medium almost as alkaline as some of the *japonicum* strains.

These results would certainly indicate that the fermentation test with glucose or galactose and under conditions similar to those followed in these experiments would not serve to give a distinct separation of organisms of these two species of legume bacteria. It would seem that this test alone is hardly sufficient to

serve as a differential character in studies with these two species. It is entirely possible that after further studies of the physiological characteristics of these organisms such a test, when supplemented by others, will prove of distinct differential value and aid considerably in the study of the legume bacteria.

SUMMARY AND CONCLUSIONS

Twenty-three strains of *Rhizobium meliloti* and twelve strains of *Rhizobium japonicum* were studied to ascertain the constancy of their fermentative characteristics and also the extent of the fermentative variations among individual strains of the same species. These organisms were grown in yeast-water-glucose and yeast-water-galactose media in five or more consecutive tests. The change in hydrogen-ion concentration in the media was taken as a measure of the fermentative ability of the strain of organisms in that medium.

The results indicate that the fermentative powers of individual strains of these bacteria were approximately the same in the various consecutive tests, and that the fermentative ability of a particular strain of organisms is a comparatively constant character.

The data also indicate clearly that there are large variations in the fermentation characters of different strains of the same species of legume bacteria. Some of the *meliloti* strains produced a distinctly alkaline reaction in the media and others produced a strongly acid reaction in media of the same composition. Some strains made the media a hundred times as acid as other strains of the same species. Some of the *japonicum* strains produced a very slightly alkaline reaction in the media whereas others produced a reaction almost ten times as alkaline.

The various strains of *Rhizobium meliloti* fermented glucose and galactose with about the same comparative results. On the other hand, the strains of *Rhizobium japonicum* did not appear to ferment these two sugars in the same manner.

The majority of the *japonicum* strains produced a much more alkaline reaction than the majority of the *meliloti* strains, but this difference was not true for all individual strains.

These results indicate that the fermentation test with glucose and galactose and under conditions similar to those followed in these experiments would not serve to give a distinct separation of organisms of these two species of legume bacteria.

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A COMPARISON OF SOME NODULE FORMING AND NON-NODULE FORMING LEGUMES FOR GREEN MANURING¹

LEWIS T. LEONARD AND H. R. REED²

Bureau of Chemistry and Soils, U. S. Department of Agriculture

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The customary assumption that leguminous crops are best for green manuring is largely based on the known ability of such crops to fix nitrogen from the air in conjunction with certain soil bacteria. The fact that such a fixation is taking place is manifested by the presence of characteristic nodular growths on the roots. The wide natural distribution of the organisms which produce these nodules and help fix nitrogen is responsible for the quite general presence of these growths. The absence of nodules from the roots of legumes is not always an indication that the plant and bacterium are incapable of forming a beneficial alliance, because the proper strain of the bacterium may be absent from the soil; but there are, however, plants classified as legumes which apparently never produce these nodules. A list of some of these plants given by Leonard (5) includes species of *Cassia* some of which are indigenous to the United States and on which it has been possible to make many observations. Since there are nodule-bearing species included in the genus *Cassia* it is quite desirable to mention that this work deals with *Cassia occidentalis* and *Cassia tora*. For these two legumes we have not found any authentic reports which show that they have ever been found with nodules on their roots in this country. Keuchenius (4) reports the presence of nodules on the roots of *Cassia occidentalis*, yet correspondence in 1923, 1926, and 1927 with the Institut voor Plantenziekten through Dr. Carl Hartley and the Algemeen Proefstation voor den Landbouw, both of Buitenzorg, Java, through the Office of Foreign Seed and Plant Introduction, Bureau of Plant Industry, U. S. Department of Agriculture, has revealed no evidence to support the statement that nodules occur on either this species or on *Cassia tora*.

The absence of the ability to form nodules does not preclude the possibility of the plant fixing nitrogen, since there is some evidence in the work of Friesner (3) and of Feher and Bokor (2) that organisms inhabit the swollen roots of the honey locust, *Gleditsia triacanthos*, and these may have a function similar to that of the nodule bacteria. Roots of the *Cassia* species under discussion are

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² Bacteriologist and formerly assistant agronomist, respectively, in the U. S. Department of Agriculture.

usually black or greenish yellow and rather thick. Information somewhat opposing this theory is given by Nobbe, Schmid, Hiltner, and Hotter (7) and by McDougall (6), all of whom ascribe lack of nodules on the roots of certain members of the sub-family *Caesalpinaceae* to the presence of thick-walled root hairs which prevent the entrance of the organisms.

Whether nodules are produced or not, whether nitrogen fixation takes place or not, both of these species of *Cassia* have been employed in green manuring experiments. The most favorable report comes from Allan (1), in which he states that a uniformly greater wheat crop over a period of 10 years was obtained after crops of *C. occidentalis* than after crops of *Crotalaria juncea*. Van Helten (9) and Rant (8) employed these two species of *Cassia* in his experimental work with green manuring crops.

OUTLINE OF EXPERIMENTAL WORK

For the purpose of determining the green manuring value of *C. occidentalis* and *C. tora*, seeds of these species and of other legumes were planted May 27, 1927, at the Coastal Plain Experiment Station, McNeill, Mississippi. The soil on which these were sown is classified as Orangeburg sandy loam. It is somewhat acid, low in nitrogen, fairly uniform in texture, and practically level. Each kind of seed was planted in rows 44 inches apart, 66 feet long, on $\frac{1}{16}$ -acre plots. An aisle 44 inches wide was provided as a dividing line between the plots. In addition to the seeded plots three unseeded ones were laid out for comparison. The crops, plot numbers, rotation, and rates of seeding are given in table 1.

The plots were located as shown in the following diagram:

North					East
5	4	3	2	1	
8	7	6			

Leguminus crops

The crops of the 1927 summer period grew well, particularly *C. tora*, which on August 19 practically hid the soil in the 44-inch rows; *Sesbenia macrocarpa*, which was 10 feet high at this time; and *Crotalaria spectabilis* which gave evidence of a heavy crop. No nodules were found on the roots of the two species of *Cassia* but all other legumes in this experiment showed nodulation; *S. macrocarpa* roots were especially well laden with nodules. On September 24 these crops were harvested from half of each plot, one-twentieth of an acre, for it was planned to turn under not only stubble but also total crop. The data obtained from these crops are given in table 2.

It is apparent that the outstanding crop yields were produced by *C. tora*

and *Crotalaria spectabilis*. Table 2 indicates that these legumes were also superior to the others in percentage of nitrogen. Ootootan soybeans did not compare favorably with the other crops either with respect to nitrogen or amount of crop produced.

All nitrogen analyses were made on dry tops of the plants. Roots were not included, since their effect should be evidenced by subsequent indicator crops.

TABLE 1
Arrangement of crops

PLOT NUMBER	1927 SUMMER CROP	ACRE RATES OF SEEDING 1927 SUMMER CROPS	1927-28 1928-29 WINTER CROPS	1928 SUMMER CROPS
		<i>pounds</i>		
1	<i>C. occidentalis</i>	100	Oats	Corn
2	None	...	Oats	Corn
3	<i>C. tora</i>	60	Oats	Corn
4	None	...	Oats	Corn
5	<i>Sesbania macrocarpa</i>	65	Oats	Corn
6	Ootootan soybean	80	Oats	Corn
7	None	...	Oats	Corn
8	<i>Crotalaria spectabilis</i>	85	Oats	Corn

TABLE 2
Crop yields and analyses of legume crops

PLOT NUMBER	CROP	MOISTURE IN GREEN HAY	ACRE YIELD OF DRY HAY	N* IN DRY HAY	N IN AN ACRE OF HAY
		<i>per cent</i>	<i>pounds</i>	<i>per cent</i>	<i>pounds</i>
1b†	<i>Cassia occidentalis</i>	52.5	3,110	1.50	46.6
3b	<i>Cassia tora</i>	62.5	4,587	1.68	77.1
5b	<i>S. macrocarpa</i>	62.5	3,945	1.20	47.3
6b	Soybean	70.0	2,131	1.60	34.1
8b	<i>Crotalaria spectabilis</i>	77.5	4,296	1.65	70.9

* Analyses made by Daniel Ready.

† Designation given to part of plot from which crops were removed.

Nonlegume indicator crops

Oats were planted on all plots, October 18, 1927, at the rate of 2 bushels an acre. On March 12, 1928, it was noticed that these oats had made the best growth on the *S. macrocarpa* plot; they were light green on the *Cassia* plots and dark green on all other plots. Hogs grazing on the oats apparently preferred material from the plots that had been fallowed. A complete report of the condition of the crop at this time is given in table 3.

The 1927-28 crops of oats from the fallow plots were superior in most respects to the crops from the other plots. This may be due to treatment, although the amount of material added to them was small and consisted only of young plants

TABLE 3
Condition of oats crop, March 12, 1927

PLOT	LEGUME PLOWED UNDER	HEIGHT OF OATS	STOOLING	COLOR
		inches		
1a	<i>Cassia occidentalis</i>	6	Little	Light green
1b	<i>Cassia occidentalis</i> stubble	5	Little	Light green
2	None	6	Medium	Dark green
3a	<i>Cassia tora</i>	7	Medium	Light green
3b	<i>Cassia tora</i> stubble	6	Little	Light green
4	None	8	Much	Dark green
5a	<i>Sesbania macrocarpa</i>	11	Much	Dark green
5b	<i>Sesbania macrocarpa</i> stubble	7	Medium	Dark green
6a	Otootan soybean	7	Medium	Dark green
6b	Otootan soybean stubble	6	Little	Dark green
7	None	7	Medium	Dark green
8a	<i>Crotalaria spectabilis</i>	9	Much	Dark green
8b	<i>Crotalaria spectabilis</i> stubble	6	Medium	Dark green

TABLE 4
Data on indicator crops following legumes

	OATS 1927-28			OATS 1928-29	CORN 1928, YIELD PER ACRE	
	Average height 4/30/28	Hay yield per acre	N in oat hay	Hay yield per acre	Stover	Grain
	inches	pounds	per cent	pounds	pounds	pounds
<i>Cassia occidentalis</i> crop	34	2,560	0.80	800	2,320	880
<i>Cassia occidentalis</i> stubble	33	2,475		720	2,325	450
None	42	4,987	1.16	1,150	1,788	773
<i>Cassia tora</i> crop	38	3,000	1.05	1,200	2,350	750
<i>Cassia tora</i> stubble	34	2,138		920	2,182	515
None	48	4,220	1.27	1,050	1,900	520
<i>Sesbania macrocarpa</i> crop	48	3,500	0.94	720	1,842	474
<i>Sesbania macrocarpa</i> stubble	43	3,600		760	2,320	640
Otootan soybean crop	40	2,860	1.07	1,100	2,111	888
Otootan soybean stubble	36	2,093		900	1,455	636
None	46	3,600	1.15	1,200	1,700	802
<i>Crotalaria spectabilis</i> crop	45	3,460	0.87	1,040	1,879	758
<i>Crotalaria spectabilis</i> stubble	38	3,218		740	2,030	636
Averages:						
Crops (nodule bearing)	44	3,273	0.96	953	1,944	707
Stubble (nodule bearing)	39	2,970		800	1,935	637
Crops (Cassias)	36	2,780	0.93	1,000	2,335	815
Stubble (Cassias)	34	2,306		820	2,254	483
Crops (all legumes)	41	3,076	0.95	972	2,100	750
Stubble (all legumes)	37	2,705		808	2,062	575
None	45	4,269	1.19	1,133	1,796	698

of *Richardsonia scabra* and cockle burs which grew during the periods the soil was not cultivated. The yield of oat hay was not only higher in the fallow plots but this hay contained more nitrogen.

The same general trend is noted in the oat crop harvested in 1929, but the yields and the differences between yields are much smaller. In only one case in either of the oat harvests is the crop following the stubble greater than that following the corresponding crop turned under, and this seems significant because it is constant in both the 1928 and 1929 figures for *Sesbania macrocarpa* plots. The same condition obtains in the corn data. It is quite possible that the proximity of pine trees to the part of the plot into which the whole crop was plowed or the abundance of cellulosic material which occurs in the plants of *Sesbania* was responsible for this condition.

Data on corn stover do not present very significant correlated differences but the corn grain yields follow the same general trend of the preceding and succeeding oat crops.

SUMMARY

In a comparison of legumes not bearing nodules with legumes bearing nodules in a green manuring experiment at McNeill, Mississippi, on slightly acid Orangeburg sandy loam, *Cassia tora*, one of the former, gave the greatest yield of dry hay, whereas Ootootan soybeans, one of the latter, gave the least yield of dry hay.

The first indicator crop of oats was light green on the *Cassia* plots whereas it was dark green on the other plots. The oats from the fallow plots, for some reason not definitely determined, were superior in nitrogen, weight, and height. A subsequent crop of oats grown on the plots during the next winter season followed the same general trend in weight.

The second indicator crop, which was corn, gave data on stover that were not very consistent and on corn grain that practically followed the same crop weight trend as the oats.

Turning under the whole crop of legumes gave better results with indicator crops than did just stubble. Considering the data as a whole, no decided differences are shown between the after-effects of noded or non-noded legumes.

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NON-RECIPROCAL CROSS-INOCULATION OF LEGUME NODULE BACTERIA¹

O. H. SEARS AND F. M. CLARK²

University of Illinois Agricultural Experiment Station

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Until recently 11 groups of legume nodule bacteria were recognized, based upon their tendency to produce nodules upon certain species of legume plants. An interchangeability was supposed to exist between the members of the same group so far as the nodule forming function is concerned. Furthermore, this phenomenon, known as cross-inoculation, was thought to occur only between members of the same group. An additional group was suggested when Whiting, Fred, and Helz (5) reported studies with Wood's clover (*Dalea alopecuroides*) nodules bacteria in which they found that this organism did not produce nodules upon alfalfa and sweet clover plants and that the Wood's clover plant was not infected by bacteria belonging to other cross-inoculating groups. No report is given of any trials with the Wood's clover organism in which attempts were made to inoculate legumes other than alfalfa and sweet clover.

Leonard (2) and Sears and Carroll (3) have shown that the cowpea group of organisms cannot be considered entirely separate from the soybean bacteria since there is some interchangeability between the two groups. The latter workers have concluded, however, that this interchangeability is not complete, since some cowpea nodule bacteria have been found which failed to produce nodules on the soybean plant. In other words, cross-inoculation between cowpea and soybean nodule bacteria is not completely reciprocal.

Stevens (4) and Wright (6) have found that it is possible to divide the groups of nodule bacteria into strains of biotypes on the basis of serological and cultural and physiological characteristics. The studies of Sears and Carroll (3) indicate that strains of cowpea bacteria apparently differ also in their capacity to produce nodules on the soybean plant.

WOOD'S CLOVER NODULE BACTERIA INFECT THE GARDEN BEAN

When Illinois farmers became interested in the use of Wood's clover for soil improvement, the problem of inoculation naturally presented itself. Conse-

¹ Contribution from the division of soil biology, department of agronomy. Published with the approval of the director.

² Assistant professor and formerly assistant in soil biology, respectively.

quently, inoculation studies with this legume were undertaken and as a result it was found that the Wood's clover plant did not produce nodules when inoculated with the nodule bacteria from any of the common legumes.

In order to establish the fact that this legume does not belong to any of the known cross-inoculation groups, it seemed necessary to conduct additional tests in which legumes representing each group were inoculated with the Wood's clover nodule organism. As a result of such studies, it was found that garden and navy bean plants produced nodules when inoculated with the Wood's clover nodule bacteria. In addition, it was found that a culture isolated from the garden or navy bean nodule, and which had resulted from the use of a Wood's clover culture, infected the Wood's clover plant. No legumes studied belonging to other cross-inoculating groups were infected by the Wood's clover nodule organism.

TABLE 1
Cross-inoculation with Wood's clover nodule bacteria

CULTURE	LEGUME	NODULATION
Sweet clover.....	Wood's clover	—
Red clover.....	Wood's clover	—
Soybean.....	Wood's clover	—
Cowpea.....	Wood's clover	—
Garden bean.....	Wood's clover	—
Navy bean.....	Wood's clover	—
	Wood's clover	+
	Sweet clover	—
	Red clover	—
Wood's clover.....	Soybean	—
	Cowpea	—
	Garden bean	+
	Navy bean	+

EXPERIMENTAL METHODS

The conditions under which cross-inoculation studies are conducted determine the reliability of the results obtained. Consequently, great care was used to prevent the probability of error due to contamination from any source. The plants were grown in a greenhouse devoted exclusively to inoculation studies, and into which no soil was allowed to be taken. As a result, the controls were uniformly free from nodules. The cultures used were obtained from our own isolations and from transfers obtained from other investigators.

In order to be sure that mixed cultures were not being used, single cell isolations were made in addition to the usual tests employed in pure culture studies.

In addition to the technique described by Sears and Carroll, the method of Garman and Didlake (1) was used.

The data presented in table 1 confirm the results of Whiting, Fred, and

Helz in that it was impossible to produce nodules upon the Wood's clover plant by inoculation with the nodule bacteria of any of the common legumes. However, it was found that cultures of the Wood's clover bacteria were able to infect plants in the garden bean group.

Previously it has been assumed that if the nodule bacteria of one kind of plant infect another, their nodule organisms are mutually interchangeable. Sears and Carroll (3) reported, however, that such a condition does not always obtain in the case of certain cowpea and soybean organisms, and in later studies they found that the cowpea plant is capable of working in symbiosis with bacteria from the nodules of a great number of legumes whose nodule organisms do not in all cases cross-inoculate among themselves. Here is a

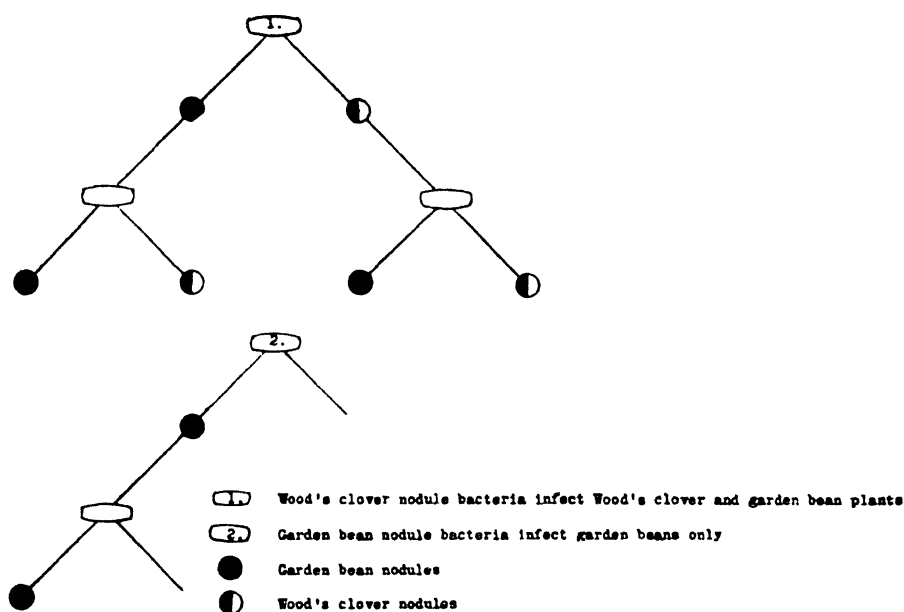


FIG. 1. RELATION BETWEEN GARDEN BEAN AND WOOD'S CLOVER NODULE BACTERIA

case in which nine garden bean cultures failed to produce nodules upon the Wood's clover plant, whereas seven cultures of Wood's clover bacteria infected the navy and garden bean plants. It would appear, therefore, that the interchangeability of nodule bacteria, as far as the nodule forming function is concerned, is not necessarily reciprocal in the case of Wood's clover and the members of the garden bean group.

It was of interest to find, however, a peculiar root development in some instances where ordinary garden or navy bean inoculation had been applied to Wood's clover plants. A knotted condition appeared upon the roots, which suggested the possibility of infection, but a number of attempts at isolation of nodule bacteria from these roots resulted in failure. This unusual root development was not observed under any other conditions.

Naturally, the question then arose as to whether bacteria isolated from the nodules on the garden or navy bean, and which had resulted from infection by Wood's clover bacteria, would still have the ability to infect Wood's clover plants. Sixteen such cultures were isolated and subsequently tested upon both garden beans and Wood's clover and in all cases with positive results. It appears, therefore, that plant passage did not alter the organism so far as its ability to form nodules on Wood's clover is concerned. Furthermore, the agar plate colonies and agar slant growth were typical of the Wood's clover organism.

Figure 1 shows diagrammatically the methods and results which are typical of those obtained from the use of seven Wood's clover and nine garden bean cultures. When obtained from picked colonies after repeated platings, as well as from direct isolation from nodules, the cultures were uniformly consistent in their behavior with regard to infecting Wood's clover and garden bean plants.

TABLE 2
Nitrogen fixation by garden bean plants

CULTURES		NITROGEN†			
Number	Kind	Tops	Roots	Total	Fixed
		mgm.	mgm.	mgm.	mgm.
1	Wood's clover	176.1	94.7	270.8	90.8
43	Wood's clover	163.3	106.0	269.3	89.3
1	Garden bean	300.1	95.9	396.0	216.0
30	Garden bean	263.3	111.5	374.8	194.8
57*	Garden bean	148.6	103.4	252.0	72.0
10*	Garden bean	157.4	98.4	255.8	75.8
	Check	110.4	69.6	180.0

* Cultures 10 and 57 were isolated from garden bean nodules which resulted from infection caused by Wood's clover nodule bacteria.

† Average of three replicates.

Since the bacteria that were obtained from widely different sources gave similar results, it seemed unlikely that this phenomenon was the result of impure cultures. The additional fact that cultures resulting from single cell isolations exhibited the same characteristics lends further evidence to prove a non-reciprocal interchangeability of garden bean and Wood's clover nodule bacteria.

Since recent investigations have shown that strains of legume nodule organisms differ in their capacity to fix atmospheric nitrogen and since two unlike organisms had produced nodules upon the garden bean, it seemed desirable to determine the comparative effectiveness of Wood's clover and garden bean nodule bacteria for nitrogen fixation. The results of such a test are reported in table 2.

Considering the nature of the experiment, the data are surprisingly consistent. The amounts of nitrogen fixed by the two cultures of Wood's clover

nodule bacteria are practically the same and differ but little from those found in the plants infected by cultures 10 and 57, which were isolated from garden bean nodules resulting from the use of Wood's clover bacteria.

Although it would not have been surprising had the two garden bean cultures differed in their nitrogen fixing ability, the results obtained from their use were very similar. They differed greatly, however, from the other cultures, which fixed an average of 82 mgm. per jar when functioning symbiotically with the garden bean plants. The garden bean plants infected by the original garden bean cultures contained 205.4 mgm. more nitrogen than the controls.

From these data, it is evident that even though abundant nodule formation resulted from the use of Wood's clover nodule bacteria on garden bean plants, the amount of nitrogen fixed was less than one-half as much as that fixed under conditions in which the garden bean plants were infected by garden bean bacteria. It is apparent, therefore, that in this case, at least, the degree of nodulation cannot be taken as an index of nitrogen fixation.

The appearance of the plants before harvest clearly showed that those inoculated with the garden bean nodule organism were getting more nitrogen than any of the other plants. They were dark green and had a healthy and thrifty appearance, whereas the crop in jars to which Wood's clover organisms had been added looked but little better than the check. In fact, even up to harvest time, there was some doubt as to whether nodulation had occurred. Examination at time of harvest showed an abundant nodule formation even though the plants were yellow and unthrifty.

SUMMARY

1. The nodule organisms from none of the common legumes studied ordinarily produced nodules on the Wood's clover plant.
2. Pure cultures of Wood's clover nodule bacteria produced nodules upon garden and navy bean plants.
3. Pure cultures of organisms isolated from the garden or navy bean infected Wood's clover, provided the bean nodules resulted from infection by Wood's clover nodule bacteria.
4. When judged by nitrogen fixation, the bean cultures were more effective upon the bean plant than the Wood's clover cultures, even though each culture produced abundant nodule development.

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RATE OF LOSS OF REPLACEABLE POTASSIUM BY LEACHING

O. C. MAGISTAD

Arizona Agricultural Experiment Station

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How rapidly is replaceable potassium lost from a soil by leaching? In the studies of fertilizer practice, of soil deterioration, and of alkali land formation and reclamation it is often desirable to have additional data on this point. Lysimeter experiments have attempted to serve the need, but the results cannot be extrapolated for higher rainfalls nor can they be carried over to other soil types, or even to the same soil when fertilized with potassium salts. This paper records several leaching experiments and shows that for any particular soil the rate of loss can be represented by a mathematical expression.

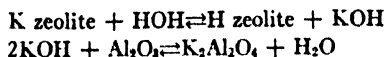
EXPERIMENTS WITH ARTIFICIALLY PREPARED POTASSIUM ZEOLITE

The plan of the present study was to leach artificial zeolites and soils of known replaceable potassium content with increments of carbon-dioxide-free water. The quantity of potassium removed in each increment was determined and an equation was obtained to fit the experimental data. Because the small amount of work on this subject by earlier authors was expressed by a different mathematical equation than that obtained by the author, all of this material has been reserved for the section "Discussion."

In the first experiments a sample of 1.575 gm. air-dry potassium zeolite was used. This zeolite contained 14.95 per cent K_2O on the air-dry basis and 21.66 per cent on the water-free basis. The sample therefore contained 5 milliequivalents potassium. The zeolite was packed in a small percolation tube between layers of washed and acid-digested asbestos, and leached with distilled, carbon-dioxide-free water at a temperature of about $28^{\circ}C$. Increments of leachate were collected, at first in 25-cc. quantities and later in larger amounts, and titrated for KOH with 0.02 N H_2SO_4 , using paranitrophenol as an indicator. Where the increments of leachate were greater than 300 cc. they were concentrated by boiling before titration. The rate of percolation was approximately 50 cc. an hour. The condensed data are given in table 1.

The last three columns of table 1 contain figures used in the mathematical treatment on leaching. This subject will be considered later.

When potassium zeolite is leached the chemical reactions can be represented by the following equations:



Calculations and data presented in a former paper (11) indicate that more than 99 per cent of the potassium hydroxide arising from hydrolysis of the zeolite is converted into potassium aluminate. A check on the accuracy of the titration can therefore be made by analyzing the solution for aluminum. Such an analysis was made on the combined percolates 21, 22, and 23, and 0.0244 gm. Al_2O_3 was obtained. The titrated value was 24 cc. or 0.48 milli-equivalents. This is equivalent to 0.0245 gm. Al_2O_3 . It is thus seen that almost a perfect check was obtained.

TABLE 1
Loss of potassium by leaching from a synthetic zeolite containing 5 milli-equivalents replaceable potassium

NUMBER OF PERCOLATE	CUM. VOLUME	CUM. TITRATION 0.2 N H_2SO_4	y = LOSS K IN MILLI-EQUIVALENTS	A - y	log(A - y)	log($\frac{A}{A-y}$)
	cc.	cc.				
4	100	8.43	0.168	4.832	0.684	0.015
6	135	10.64	0.213	4.787	0.680	0.019
8	205	14.30	0.286	4.714	0.673	0.026
10	305	19.00	0.380	4.620	0.665	0.034
12	455	24.60	0.492	4.508	0.654	0.045
14	655	30.70	0.614	4.386	0.642	0.057
16	1,000	38.20	0.764	4.236	0.627	0.072
18	1,490	48.60	0.972	4.028	0.605	0.094
20	2,500	68.60	1.372	3.628	0.560	0.139
22	3,500	80.90	1.618	3.382	0.529	0.170
24	4,500	94.60	1.892	3.108	0.492	0.206
26	5,500	105.60	2.112	2.888	0.461	0.238
28	7,500	122.60	2.452	2.548	0.406	0.293
30	9,500	138.00	2.700	2.240	0.350	0.349
32	11,500	151.70	3.034	1.966	0.294	0.405
34	13,500	162.80	3.256	1.844	0.266	0.433
36	15,500	172.60	3.452	1.548	0.190	0.509
38	17,500	180.50	3.610	1.390	0.143	0.556
40	19,500	187.00	3.740	1.260	0.100	0.599
42	21,500	189.40	3.788	1.212	0.083	0.615
44	23,500	192.00	3.840	1.160	0.065	0.635

In the second experiment in which artificial zeolites were used, a quantity equivalent to 10 milli-equivalents replaceable potassium was used. The procedure was similar to that used in the first experiment. A condensed summary of the data obtained is given in table 2.

The data given in table 2 will be treated mathematically under "discussion."

EXPERIMENTS WITH SOILS

In the first experiment with soils a fine sandy loam from the new university farm was used. This soil was first treated with dilute hydrochloric acid to remove all carbonates. It was then leached with a potassium chloride solution

containing a small quantity of potassium hydroxide, followed by potassium chloride alone. The soil was finally washed with water until only a very slight test for chloride in the leachate could be obtained.

The base-exchange capacity of this modified soil was determined and found to be 3.262 milli-equivalents potassium and 0.78 milli-equivalents calcium plus magnesium for each hundred grams.

Five hundred grams of this soil was packed into a small Oldberg percolator and percolated with water using 100- to 150-cc. aliquots. The percolates were analyzed for potassium. The rate of percolation was about 10 cc. an hour

TABLE 2

Loss of potassium by leaching from a synthetic zeolite containing 10 milli-equivalents replaceable potassium

NUMBER OF PERCOLATE	CUM VOLUME	CUM. TITRATION 0.2 N H ₂ SO ₄	y = LOSS K IN MILLI- EQUIVALENTS	A - y	log (A - y)	log ($\frac{A}{A - y}$)
	cc.	cc.				
3	80	16.20	0.324	9.676	0.9857	0.0143
6	185	23.10	0.462	9.538	0.9795	0.0205
9	337	33.45	0.669	9.331	0.9699	0.0301
12	617	48.95	0.979	9.021	0.9552	0.0448
15	1,267	77.55	1.551	8.449	0.9268	0.0732
18	1,867	98.05	1.961	8.039	0.9052	0.0948
21	2,467	116.65	2.333	7.667	0.8846	0.1254
24	4,000	147.75	2.955	7.045	0.8479	0.1521
27	5,500	177.15	3.543	6.457	0.8100	0.1900
30	7,000	201.35	4.027	5.973	0.7762	0.2238
33	8,500	220.15	4.403	5.597	0.7480	0.2520
36	10,000	236.85	4.737	5.263	0.7212	0.2788
39	12,000	256.15	5.323	4.677	0.6699	0.3301
42	15,000	277.95	5.559	4.441	0.6475	0.3525
45	18,000	302.85	6.057	3.943	0.5958	0.4042
48	21,000	327.55	6.551	3.449	0.5377	0.4623
51	24,000	342.55	6.851	3.149	0.4982	0.5018
54	27,000	360.25	7.205	2.795	0.4464	0.5536
57	30,000	366.45	7.329	2.671	0.4267	0.5733
60	33,000	369.35	7.387	2.613	0.4171	0.5829

until about 800 cc. had passed, when the rate dropped to 30 cc. a day. The data obtained are given in table 3.

In the second experiment a modified Carrington silt loam soil was used. This soil is described as soil 5 in Arizona Agricultural Experiment Station Technical Bulletin 22, and contained 7.13 milli-equivalents replaceable potassium in 100 gm. soil. Five hundred grams of this soil was leached in the same manner as the university farm soil and the leachings were collected and analyzed. The data obtained are given in table 4.

In a third experiment the same modified Carrington silt loam was used as in experiment 2. To it was added precipitated calcium carbonate to equal 2

per cent of its weight. Five hundred grams of this calcareous soil was placed in a percolator and kept in a moistened condition for 2 months before leaching

TABLE 3
Loss of potassium by leaching from a modified soil from the University farm

NUMBER OF PERCOLATE	CUM. VOLUME	y = LOSS K IN MILLI- EQUIVALENTS	A-y	log(A-y)	$\log\left(\frac{A}{A-y}\right)$
	cc.				
1	70	0.77	15.54	1.191	0.021
2	170	1.30	15.01	1.176	0.036
3	278	1.71	14.60	1.164	0.048
4	424	2.02	14.29	1.155	0.057
5	568	2.26	14.05	1.148	0.064
7	859	2.65	13.66	1.135	0.077

TABLE 4
Loss of potassium by leaching from a modified Carrington silt loam

NUMBER OF PERCOLATE	CUM. VOLUME	y = LOSS K IN MILLI- EQUIVALENTS	A-y	log(A-y)	$\log\left(\frac{A}{A-y}\right)$
	cc.				
1	114	1.71	33.94	1.531	0.021
2	210	2.66	32.99	1.518	0.034
3	329	3.34	32.31	1.509	0.043
4	465	3.88	31.77	1.502	0.050
5	597	4.47	31.18	1.494	0.056
6	725	4.89	30.76	1.488	0.064
7	875	5.48	30.17	1.479	0.073

TABLE 5
Loss of potassium by leaching from a calcareous Carrington silt loam

NUMBER OF PERCOLATE	CUM. VOLUME	y = LOSS K IN MILLI- EQUIVALENTS	A-y	log(A-y)	$\log\left(\frac{A}{A-y}\right)$
	cc.				
1	110	0.47	35.18	1.546	0.006
2	220	1.05	34.60	1.539	0.013
3	384	1.61	34.04	1.532	0.020
4	468	2.12	33.53	1.525	0.027
5	589	2.72	32.93	1.518	0.034
6	713	3.27	32.38	1.510	0.042
7	829	3.73	31.92	1.504	0.048
8	959	4.25	31.40	1.497	0.055

was begun. The leachings were collected and analyzed as before. The data so obtained are given in table 5.

The data presented in tables 3, 4, and 5 will be treated mathematically under "Discussion."

DISCUSSION

Schreiner and Failyer in 1906 (17) published a paper describing the absorption of potassium by soils and its subsequent removal by leaching. They apparently imply, but do not seem to state definitely, that absorbed potassium is removed according to the differential equation.

$$\frac{dy}{dv} = K (A - y)$$

However, Patten and Waggaman (14), who later carried on similar studies, say, "Thus we have shown that the removal curves for bicarbonate, carbonate, chlorine, potassium, and phosphate leached from soils are very similar in form," and, "The absorption of potassium from carbonate and from chloride solution is likewise described by curves similar in form, and very nearly represented by the formula $\frac{dy}{dv} = K(A - y)$, which also expresses well the rate of absorption and rate of removal of the phosphate radical, PO_4 , from soils¹ regardless of what phosphate was used to saturate the soil."

It would thus seem that Schreiner and Failyer's data on removal of potassium by leaching also follow the same differential equation. In this equation, when used to represent leaching data, y is the amount removed by v cc. of water, A is the absorptive capacity and K is a constant. Schreiner and Failyer integrate this differential equation between limits as follows:

$$\int_0^y \frac{dy}{A - y} = K \int_0^v dv \quad (A)$$

obtaining

$$\log (A - y) - \log A = -Kv \quad (B)$$

They state that since $\log A$ and K are constants to be found from the observations, Briggsian instead of Napierian logarithms may be used. Schreiner and Failyer seem to have obtained the value of A by inspection of the curve obtained by plotting the experimental data.

It was believed that the rate of loss of potassium from artificial zeolites should follow the differential equation (A). Equation (B) indicates that if the values of $\log \left(\frac{A}{A - y} \right)$ given in tables 1 and 2 are plotted as ordinates against the volume in cubic centimeters as abscissas, a straight line whose slope is K should be obtained. Because it is impractical to plot volume as given, log volume was used; equation (B) now becomes

$$\log \log \left(\frac{A}{A - y} \right) = \log K + \log V \quad (C)$$

¹ (Schreiner and Failyer) Bul. 32, Bur. Soils, U. S. Dept. Agr. p. 36. (Ref. given by Patten and Waggaman.)

To simplify operations log log paper was used and $\log \left(\frac{A}{A-y} \right)$ was plotted against V . These data and resulting curves are shown in figure 1. The curve for experiment 1 was a straight line having a slope of 0.695 and for experiment 2, 0.650. The fact that the slope is not 1.00 but a decimal shows that the data do not fit equation (C).

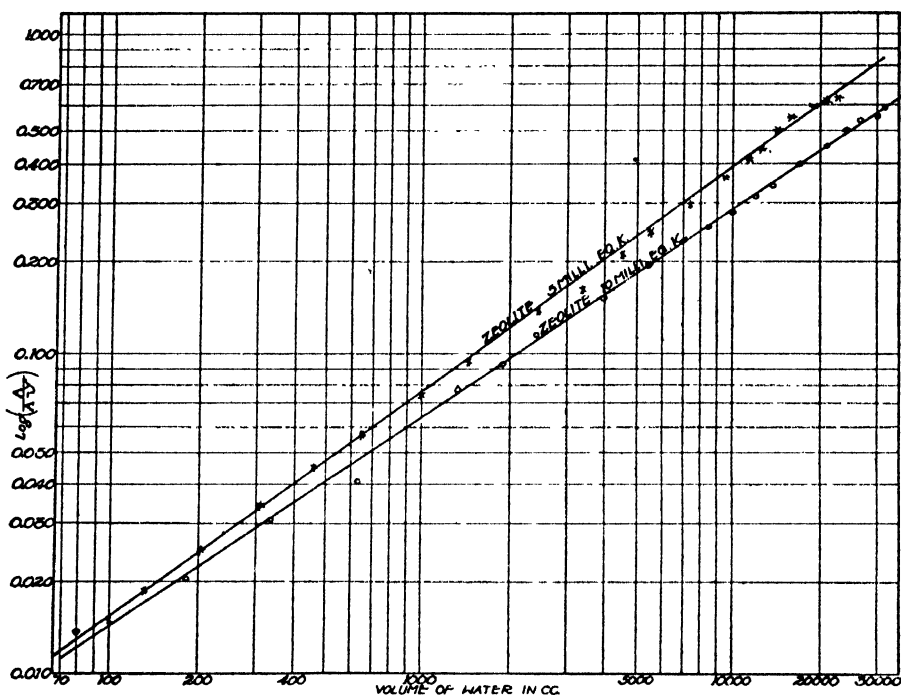


FIG. 1. LOSS OF POTASSIUM BY LEACHING FROM ARTIFICIAL ZEOLITES CONFORMS WITH THE EQUATION

$$\log \left(\frac{A}{A-y} \right) = KV^p \text{ (Value of } p \text{ about 0.65)}$$

Instead, the equations of the curves are

$$\log \frac{A}{(A-y)} = KV^{0.695} \quad (D)$$

and

$$\log \frac{A}{(A-y)} = KV^{0.650} \quad (E)$$

for experiments 1 and 2 with the artificial zeolites respectively. The values of A and K for equation (D) are 5 and 0.00061 and for equation (E) they are 10 and 0.00071.

In a similar manner the values of $\log \left(\frac{A}{A-y} \right)$ from tables 3 and 4 were plotted against the corresponding volumes in cubic centimeters on log log coordinate paper. Both curves were straight lines having slopes of 0.53 and 0.63 respectively (fig. 2). After it is found that the data can be represented by a straight line curve, and the slope of this straight line is known, the values of K

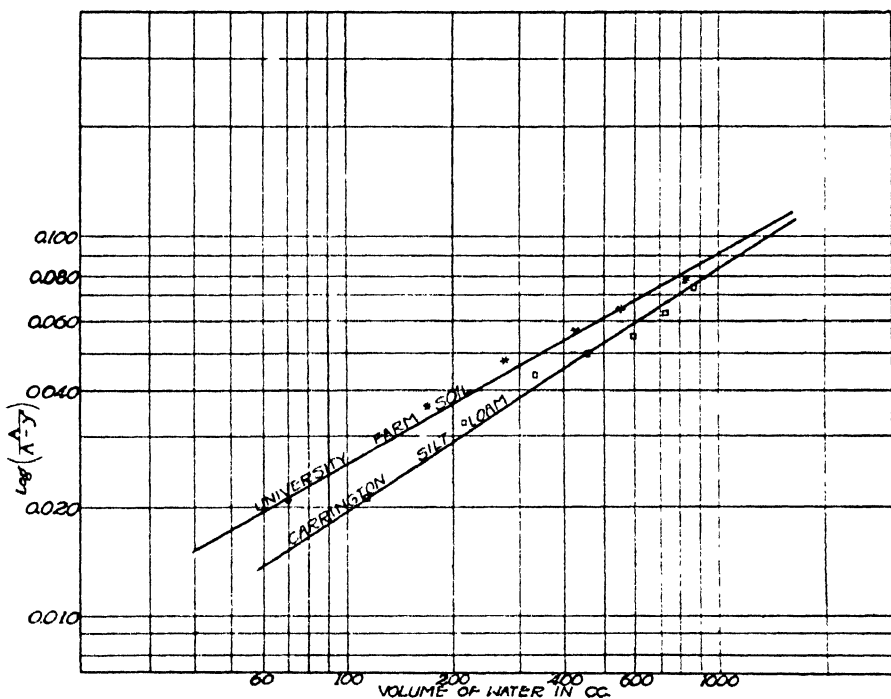


FIG. 2. LOSS OF POTASSIUM BY LEACHING FROM UNIVERSITY FARM SOIL AND CARRINGTON SILT LOAM CONFORMS WITH THE EQUATION

$$\text{Log} \left(\frac{A}{A-y} \right) = KV^p$$

can be calculated. It was thus found that the leaching equations for the university farm soil and Carrington silt loam were

$$\text{Log} \left(\frac{A}{A-y} \right) = 0.00219 V^{0.53}$$

and

$$\text{Log} \left(\frac{A}{A-y} \right) = 0.00100 V^{0.63}$$

respectively.

It must be remembered that Schreiner and Failyer obtained their value of A from inspection of the absorption curve. The maximum capacity of their soils should have been $A + Y_0$ if we let Y_0 represent the amount of replaceable potassium present before the absorption experiments began. In the author's experiments the maximum exchange capacity of the zeolite in experiment 1 is 7.96 milli-equivalents on the basis of 1 mol K_2O to 1 mol Al_2O_3 .

Let us use this value $7.96 = M^2$ where M represents the maximum capacity. Let Y_0 equal the loss from this zeolite before the leaching experiment began, or

$$Y_0 = 7.96 - 5.00 = 2.96$$

then $M = A + Y_0$. Integrating the differential equation of Spillman and Lang (18, p. 34)

$$\frac{dY}{dV} = K(M - Y)$$

between the limits as follows:

$$\int_{Y_0}^{Y_1} \frac{dY}{M - Y} = K \int_{V_0}^{V_1} dV$$

we obtain

$$\ln (M - Y_1) - \ln (M - Y_0) = -K(V_1 - V_0). \quad (F)$$

If we let $V = V_1 - V_0$, $Y = Y_1 - Y_0$ and $M = A + Y_0$ and substitute these values in equation (F), we obtain

$$\ln (A + Y_0 - Y - Y_0) - \ln (A) = -KV$$

or

$$\ln \left(\frac{A}{A - Y} \right) = KV$$

If we wish to pass to common logarithms the equation becomes

$$\log \left(\frac{A}{A - Y} \right) = K_1 V \quad (G)$$

where $K_1 = 0.4343K$.

The derivation of equation (G) would indicate that it is perfectly proper to use the quantity of exchangeable potassium at the beginning of leaching as A and to plot the values of $\log \left(\frac{A}{A - y} \right)$ against V , V being the volume of water leached through the sample during the experiment.

² The notations used by Spillman and Lang (18), are used as far as possible.

It is possible that the values of A used by Schreiner and Failyer, and by Patten and Waggaman are too small, and that this reduced value of A is responsible for their data giving a slope of 1.00 when $\log \left(\frac{A}{A-y} \right)$ is plotted against volume?

It must be remembered that the value of A used by the aforementioned authors is equivalent to M of equation (F). The fact that the authors did not consider the soil to contain any previously absorbed potassium, and that for the purpose of calculation the value of $A - Y_0$ is less than the amount present at the beginning of leaching would all indicate that their values of A are too small.

WORK OF EARLIER AUTHORS RECALCULATED

Let us recalculate the results of Schreiner and Failyer and of Patten and Waggaman in the light of present knowledge of base exchange. Two assumptions are made, first, that all the potassium present in the soil at the beginning and subsequently removed by leaching was replaceable, and second, that the soil contained normal amounts of replaceable potassium prior to the absorption experiments. The results for the non-calcareous soils are shown in figure 3; the points for any particular soil fall on a straight line curve whose equation is similar to that for preceding soils and zeolites.

The values of A , assumed quantities of replaceable potassium originally present, and of constants in the leaching equations are all given in table 6, which is a recapitulation of all leaching data in this paper.

If we exclude the calcareous soils it will be seen that the leaching equation of the remaining soils has constants of the same order. The leaching equation for zeolites is of the same type but the value of K is somewhat smaller.

Greaves, Hirst, and Lund (5, table 13) present considerable data on the rate of loss of potassium by leaching from synthetic and natural alkali soils. An inspection of their table shows that the potassium is removed in decreasing successive increments and it would appear that the data would fit some sort of logarithmic curve. Because the original amount of exchangeable potassium is not known the data cannot be tested for their conformity with leaching equations as given in this paper.

It should be pointed out that all the leaching experiments for which we have data were short time, excessive leaching, experiments. Under field conditions with more time at our disposal, it is probable that a small quantity of non-replaceable potassium becomes replaceable in the case of soils low in exchangeable potassium. In soils whose exchangeable potassium content has recently been raised, a gradual conversion of some of the replaceable potassium to non-replaceable form seems to take place.

Page and Williams (12) give excellent data on this point and the work of MacIntire and Sanders (10), Pierre and Morley (15), and Frear and Erb (4) corroborates them.

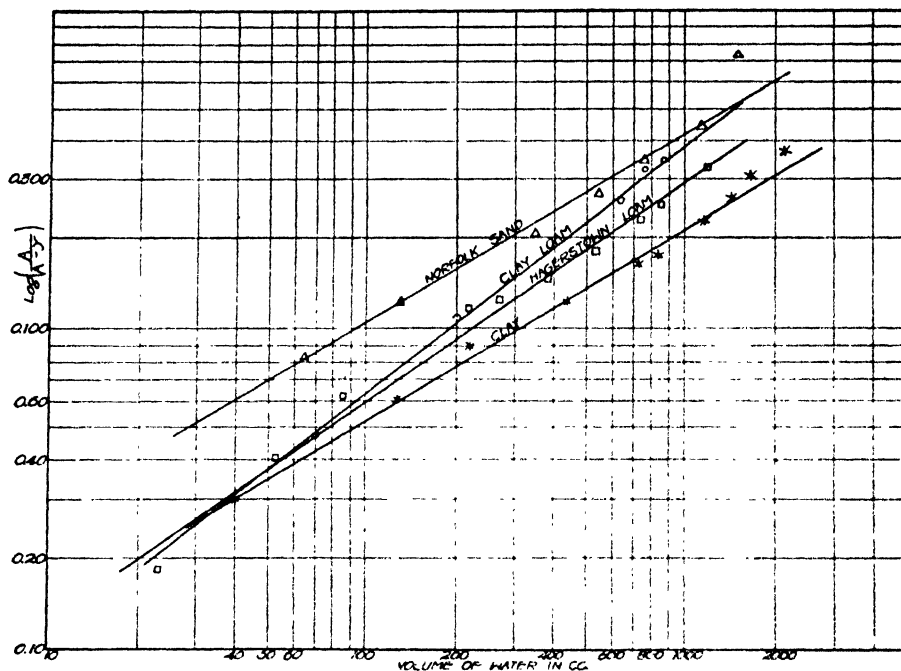


FIG. 3. LOSS OF POTASSIUM BY LEACHING FROM SOILS USED BY SCHREINER AND FAILYER AND BY PATTEN AND WAGGAMAN, AS RECALCULATED BY THE AUTHOR, ALSO CONFORM WITH THE EQUATION

$$\text{Log} \left(\frac{A}{A - y} \right) = K V^p$$

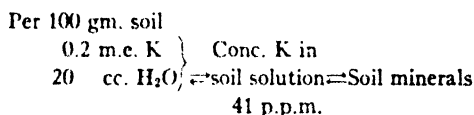
TABLE 6

A comparison of the constants in the potassium leaching equation for soils and artificial zeolites

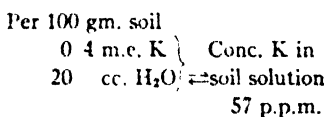
SOIL	INVESTIGATOR	REPLACEABLE K ASSUMED PRESENT PER 100 GW SOIL BEFORE ABSORPTION		ASSUMED OR KNOWN VALUE OF A	P EXPO- NENT OF V	K
		m.eq.	p.p.m.			
Artificial zeolite.....	Magistad	5.0 m.e.	0.695	0.00061
Artificial zeolite.....	Magistad	10.0 m.e.	0.650	0.00071
University farm soil.....	Magistad	16.31 m.e.	0.53	0.00219
Carrington silt loam.....	Magistad	35.65 m.e.	0.63	0.00100
Clay.....	S and F	0.050	20	910.00 p.p.m.	0.60	0.00316
Clay loam.....	S and F	0.025	10	580.00 p.p.m.	0.69	0.00295
Hagerstown silt loam.....	P and W	0.100	40	2,974.00 p.p.m.	0.68	0.00263
Hagerstown silt loam.....	P and W	1.000	400	3,334.00 p.p.m.	0.64	0.00316
Norfolk sand.....	P and W	0.500	200	1,426.00 p.p.m.	0.58	0.00760
<i>Calcareous soils</i>						
Marshall silt loam.....	P and W	1.000	400	2,648.00 p.p.m.	1.00	0.00078
Marshall silt loam.....	P and W	none	none	2,248.00 p.p.m.	0.99	0.00064
Calcareous Carrington silt loam	Magistad	35.65 m.e.	1.02	0.00005

It seems that an equilibrium of some sort exists between the potassium containing soil minerals and the soil solution on one hand, and the soil solution and the exchangeable complex on the other. If the amount of exchangeable potassium decreases to such a point that the concentration of potassium in the soil solution is markedly diminished, potassium will go into solution from the soil minerals and vice versa. Data presented in a former paper (11) show a logarithmic relationship between the amount of potassium in the soil solution and that present in exchangeable form, and later studies (1) showed that finely ground feldspar will maintain, to a greater or lesser degree, a certain concentration of potassium in the soil solution.

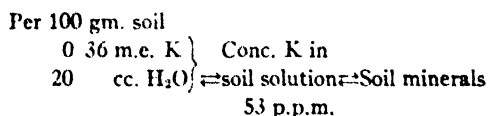
As a concrete example, let us consider a soil which contains 0.2 milli-equivalent of exchangeable potassium to each 100 gm. and contains 20 per cent of water. The soil solution will probably contain about 41 p.p.m. of K which is in equilibrium with the other soil minerals. This equilibrium can be represented thus:



Let us suppose now, that an amount of soluble potassium salt is applied which will approximately double the quantity of exchangeable potassium in the soil. The first part of the foregoing equilibrium expression will then be.



Because the soil solution now contains more K, a new equilibrium between the soil solution and the soil minerals will take place. Final equilibrium of the entire system will probably take place according to the following expression.



The net result of the fertilizer addition has been to increase the exchangeable potassium 0.16 milli-equivalents to each 100 gm. and the non-exchangeable potassium 0.04 milli-equivalents to 100 gm. This condition of equilibrium is not attained rapidly; undoubtedly several months are necessary.

EFFECT OF CaCO_3 ON POTASH LIBERATION

The leaching data for the calcareous soils, the Marshall silt loam and the limed Carrington silt loam, are shown in figure 4. It will be seen that the slope of these curves is about 1.00, which seems to be distinctive for calcareous

soils. A comparison of the leaching data on the calcareous and non-calcareous Carrington silt loams shows that the presence of calcium carbonate has decreased the rate of loss of potassium by leaching. Earlier experiments by the author (11) indicate that this decrease is caused by a decreased hydrolysis. Calcium carbonate in water and the hydrolysis of potassium zeolite both give rise to hydroxyl ions. The common ion will decrease hydrolysis, which may be the principal factor responsible for decreased potassium availability in

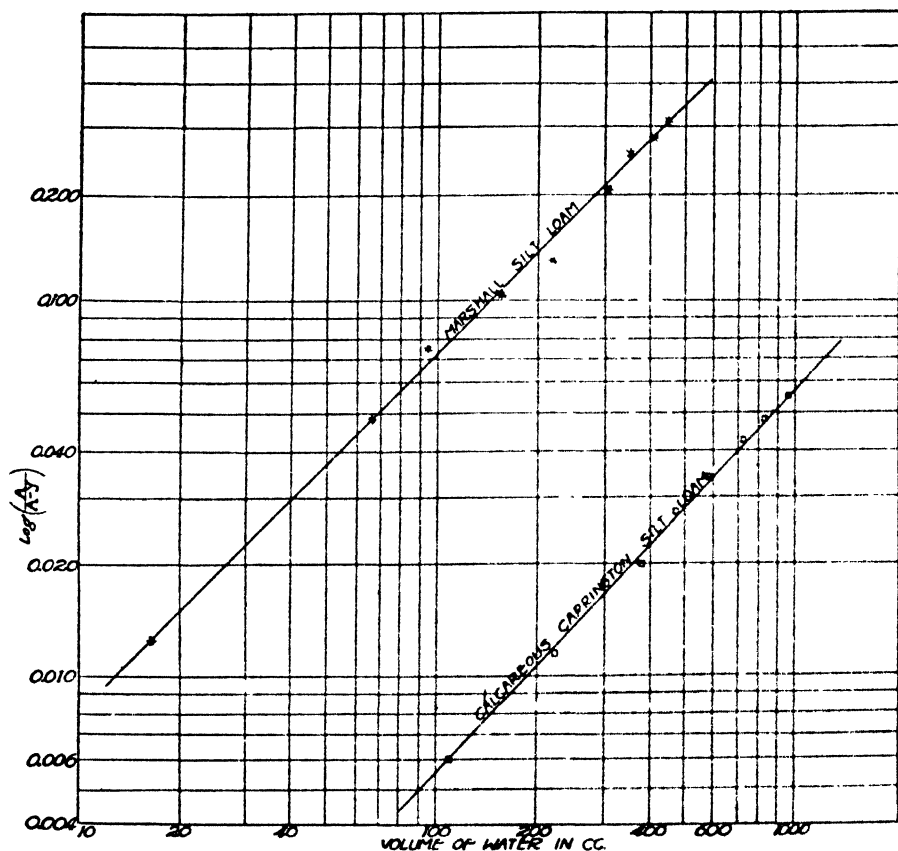


FIG. 4. LOSS OF POTASSIUM BY LEACHING FROM MARSHALL SILT LOAM AS RECALCULATED BY THE AUTHOR, AND CALCAREOUS CARRINGTON SILT LOAM, CONFORM WITH THE USUAL EQUATION BUT WITH A VALUE OF p APPROACHING 1

limed soils. When the solutions were made less alkaline by the introduction of carbon dioxide, the decrease in hydrolysis of potassium zeolite was less marked (11).

A review of the more recent literature indicates that most investigators have found applications of calcium or magnesium carbonates or oxides to decrease the liberation of potash. This is borne out by the work of MacIntire et al. (8, 9), Parker and Tidmore (13), Lyon (6), Plummer (16), Brown and

MacIntire (2), and MacIntire (7). On the other hand, Fraps (3) found that crops of corn, cotton, and sorghum took up slightly more potash from the soil after treatments with calcium carbonate or organic matter than previously.

SUMMARY

Synthetic potassium zeolites were leached with distilled water and the successive portions of leachate analyzed for potassium. The data obtained could be expressed by means of an equation.

Similar studies on a University farm soil and a Carrington silt loam gave rise to a similar equation.

The early work on leaching by Schreiner and Failyer and by Patten and Waggaman has been reviewed. Their data were recalculated in the light of modern base-exchange ideas. It was found that their data did not fit the differential equation

$$\frac{dv}{dv} = K(A - y)$$

very well, nor the resulting integral equation

$$\log(A - y) - \log A = -Kv$$

The data did seem to agree much better with an equation of the type

$$\log\left(\frac{A}{A - I}\right) = K V^p$$

where p is a fraction.

The constants in the leaching equation were fairly uniform for the non-calcareous soils. Those for the calcareous soils indicated a much lower rate of loss of potassium, whereas the constants for synthetic zeolites fell between that for calcareous and non-calcareous soils.

Although the constants in the leaching equation for potassium for widely different soils vary considerably, it seems plausible that for a particular series a single set of constants would hold fairly well and that the use of an equation containing these constants would enable one to estimate the rate of loss of potassium over periods of a few years.

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SOME CARBON-NITROGEN RELATIONS IN SOILS

WILBUR R. LEIGHTY AND EDMUND C. SHOREY¹

Bureau of Chemistry and Soils, U. S. Department of Agriculture

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All soils contain organic matter, which in turn contains carbon as an element essential to its organic character, and some of which contains nitrogen in organic combination.

This organic content results from the addition to the soil of the remains of plants, animals, or microorganisms living on or in it. In the changes that take place in the decay of such remains the proportion of carbon to nitrogen becomes less, the carbon disappearing for the most part in the form of carbon dioxide. In the original vegetable or animal remains the proportion of carbon to nitrogen is about 40 to 1, except in the case of leguminous plants with higher nitrogen content, when it may be as low as 25 to 1. In soils, however, it has been commonly held that it is about 10 to 1 and does not vary much; but it has not been apparent just to what extent this assumption of a uniform ratio of 10 to 1 is based on analytical data. Our knowledge of definite nitrogenous organic compounds in soils is not very extensive, but we know that such compounds have a low C:N ratio; for instance, arginine 5.5:1, histidine 2.6:1, xanthine 1.4:1, lysine 2.6:1, cyanuric acid 0.53:1.

In the ordinary fractionation of soil organic matter, whether by acids, alkalis, or organic solvents, it is difficult to obtain fractions that are free from nitrogen. However, on purification by precipitation or repeated solution, a considerable quantity can be obtained nitrogen free; and it is quite apparent that the organic matter of soils is made up of a mixture of compounds—some containing nitrogen and others none; and it might very well be assumed that the proportions of these two classes of compounds would vary in different soils, with a corresponding variation in the C:N ratio.

With a view to determining how far analytical data support the contention, or assumption, that this ratio of C to N in soils is fairly constant, analyses made in these laboratories for other purposes have been reviewed.

The soil samples represent 63 locations in 12 states. In most instances samples at three or more levels were taken, making 172 samples in all.

The samples were taken at points where it was proposed to carry on fertilizer experiments, and the soils were in cultivation at the time the samples were taken. The upper profile of such soils had, of course, been disturbed, but the

¹ Assistant and senior biochemists, respectively, soil fertility investigations.

division between samples was made at depths where color and texture indicated a change from one horizon to another. No horizon designations have been used; however, the depths to which the samples were taken are stated.

The total carbon was determined by combustion in oxygen, and carbon dioxide from carbonates, if present, was deducted from that obtained in combustion, the difference being stated as organic carbon.

The nitrogen was determined by the Kjeldahl method not modified to include nitrates, but should some nitrate appear in the total by the method used, the possible increase would not be significant. A nitrate (NO_3) content of 100 p.p.m. which might be considered excessive, would mean, if it were all recovered, an increase in the total N content of only 0.002 per cent.

The analytical data and calculated C:N ratios are given in table 1. The average C:N ratio given for each location is the average without weighting for variation in the depth to which samples were taken.

The first point apparent on a review of the figures in table 1 is the quite wide variation from the commonly accepted ratio of 10:1. The highest, 35.2:1 was found at the 18- to 36-inch level in the Iberia silty clay loam at Franklin, Louisiana; and the lowest, 3.5:1 at the 24- to 36-inch level in the Bladen fine sandy loam at Baldwin, Florida, with other ratios pretty well spread between these points.

The second point is that in most cases there is a regular lowering of the ratio from the top to the lowest horizon, this being the case in 42 samples out of 46 where three or more depths are represented.

There are 3 samples where the ratio is highest at the lowest depth, and 6 samples where the ratio at the top is three times the ratio at the lowest level. There are 28 locations out of the 76 where the ratio is less than 10:1; 4 where it is 10+:1; and in the remaining 44, 11:1 or above. The average of all the samples without weighting for variation in depth of samples is 10.5:1, and the average of all surface samples is 12.8:1. The average of all samples at 36 inches is 8.2:1.

Other comparisons may, of course, be made, but the figures speak for themselves in opposition to the contention that the proportion of carbon to nitrogen in average American soils is uniform.

In a series of plats at Tifton, Georgia, devoted to green manuring experiments, the analytical data are of interest in this connection. In the plats which receive green manure treatment, the figures for 1928 show a nitrogen content practically uniform, the variation being from 0.03 to 0.04 per cent. The carbon content, although not so uniform, showed no wide variation, being from 0.71 to 1.03 per cent. The C:N ratio varies from 18:1 to 27:1 with an average of 22.7:1. This offers a good example of the ratio being maintained by annual additions of fresh vegetable matter with a ratio probably in excess of 30 or 40 to 1 at a point twice that accepted as the normal.

A further example of extreme variation in the C:N ratio was found in partial analyses of samples of soil from Fontana, California. In these 6 samples the

TABLE 1

Analytical data and calculated C:N ratios of the soil samples studied

LOCATION AND SOIL TYPE	DEPTH	NITROGEN	ORGANIC CARBON	RATIO C TO N-1	AVERAGE C TO N-1
	<i>inches</i>	<i>per cent</i>	<i>per cent</i>		
Presque Isle, Me., Caribou loam	0-8	0.227	3.00	13.2	10.3
	8-12	0.141	1.55	10.9	
	12-18	0.077	0.77	10.0	
	18-36	0.071	0.50	7.0	
Presque Isle, Me., Washburn loam	0-8	0.320	4.90	15.3	
Allentown, Pa., Berkshale loam	0-8	0.176	1.81	10.2	7.8
	8-18	0.088	0.81	9.2	
	18-36	0.046	0.19	4.1	
Bridgeton, N. J., Sassafras loam	0-10	0.125	1.20	9.6	7.9
	10-20	0.031	0.23	7.4	
	20-36	0.022	0.15	6.8	
Freehold, N. J., Sassafras loam	0-10	0.143	1.66	11.6	8.0
	10-20	0.038	0.33	8.7	
	20-36	0.023	0.09	3.9	
Holmdel, N. J., Sassafras loam	0-12	0.142	1.59	11.1	9.0
	12-18	0.042	0.43	10.3	
	18-36	0.037	0.21	5.6	
Hightstown, N. J., Sassafras gravelly sandy loam	0-10	0.090	0.92	10.2	8.6
	10-18	0.038	0.32	8.4	
	18-36	0.022	0.16	7.2	
Long Island, N. Y., Sassafras sandy loam	0-10	0.116	1.61	13.8	10.6
	10-18	0.054	0.53	9.8	
	18-36	0.032	0.27	8.4	
Cape Charles, Va., Sassafras sandy loam	0-8	0.069	0.86	12.4	8.5
	8-15	0.040	0.31	7.7	
	15-36	0.039	0.22	5.6	
Cape Charles, Va., Sassafras sandy loam, 1921 field	0-7	0.075	0.91	12.1	11.0
	7-16	0.035	0.38	10.8	
	16-36	0.029	0.30	10.3	
Cape Charles, Va., Sassafras sandy loam, 1923 field	0-8	0.065	0.69	10.6	7.3
	8-18	0.026	0.19	7.3	
	18-36	0.023	0.09	3.9	
Bridgetown, Va., Sassafras sandy loam	0-8	0.052	0.51	9.8	7.2
	8-18	0.030	0.14	4.6	
	18-36	0.030	0.22	7.3	

TABLE 1—*Continued*

LOCATION AND SOIL TYPE	DEPTH	NITROGEN	ORGANIC CARBON	RATIO C TO N-1	AVERAGE C TO N-1
	<i>inches</i>	<i>per cent</i>	<i>per cent</i>		
West Norfolk, Va., Norfolk fine sandy loam.....	0-9	0.062	0.77	12.4	11.1
	9-20	0.021	0.16	7.6	
	20-36	0.021	0.28	13.3	
Portsmouth, Va., Norfolk fine sandy loam, 1921 field.....	0-10	0.091	0.65	7.1	6.3
	10-20	0.021	0.16	7.6	
	20-36	0.023	0.10	4.3	
Portsmouth, Va., Norfolk fine sandy loam, 1922 field.....	0-10	0.106	1.13	10.6	9.8
	10-18	0.028	0.25	8.9	
	18-36	0.025	0.25	10.0	
Suffolk, Va., Bladen fine sandy loam.....	0-8	0.075	0.87	11.6	8.2
	8-18	0.019	0.14	7.3	
	18-36	0.019	0.11	5.7	
Darlington, S. C., Norfolk sandy loam ..	0-8	0.026	0.49	18.1	15.4
	8-30	0.013	0.26	20.0	
	30-36	0.025	0.20	8.0	
Darlington, S. C., Norfolk coarse sandy loam, 1919 field.....	0-8	0.020	0.53	26.5	15.7
	8-20	0.009	0.11	12.2	
	20-36	0.020	0.17	8.5	
Florence, S. C., Norfolk fine sandy loam.....	0-8	0.033	0.52	15.7	13.9
	8-18	0.010	0.12	12.0	
	18-36	0.024	0.34	14.2	
Florence, S. C., Norfolk very fine sandy loam.....	0-8	0.049	1.06	21.6	15.3
	8-18	0.016	0.27	16.8	
	18-36	0.024	0.18	7.5	
Darlington, S. C., Portsmouth sandy loam.....	0-8	0.055	0.80	14.5	11.7
	8-36	0.027	0.24	8.9	
New Bern, S. C., Portsmouth sandy loam..	0-12	0.052	0.96	18.5	21.4
	12-36	0.028	0.68	24.3	
Darlington, S. C., Ruston sandy loam 1920 field.....	0-8	0.033	0.59	17.8	13.4
	8-20	0.012	0.19	15.8	
	20-36	0.015	0.10	6.6	
Fayetteville, N. C., Ruston sandy loam..	0-8	0.034	0.77	22.6	15.3
	8-18	0.009	0.14	15.5	
	18-36	0.033	0.26	7.8	
Bennettsville, S. C., Marlboro sandy loam..	0-8	0.042	1.05	25.0	15.2
	8-14	0.013	0.19	14.6	
	14-36	0.031	0.19	6.1	

TABLE 1—Continued

LOCATION AND SOIL TYPE	DEPTH	NITROGEN	ORGANIC CARBON	RATIO C TO N-1	AVERAGE C TO N-1
	<i>inches</i>	<i>per cent</i>	<i>per cent</i>		
Lucama, N. C., Marlboro sandy loam....	0-5	0.055	0.99	18.0	13.0
	12-18	0.031	0.25	8.0	
Athens, Ga., Cecil sandy loam.....	0-8	0.051	0.70	13.7	12.9
	8-36	0.027	0.33	12.2	
Shelby, N. C., Cecil sandy loam.....	0-8	0.034	0.59	17.3	14.4
	8-15	0.027	0.36	13.3	
	15-36	0.021	0.27	12.7	
Gastonia, N. C., Cecil fine sandy loam...	0-10	0.041	0.56	13.6	10.3
	10-18	0.033	0.36	10.9	
	18-36	0.018	0.12	6.6	
Lindwood, N. C., Davidson clay loam	0-7	0.066	1.12	16.9	14.8
	7-12	0.055	0.70	12.7	
New Bern, N. C., Dunbar fine sandy loam	0-8	0.065	0.98	15.0	9.6
	8-18	0.020	0.16	8.0	
	18-36	0.027	0.16	5.9	
Fayetteville, N. C., Wickham fine sandy loam	0-7	0.032	0.34	10.6	9.4
	7-14	0.035	0.29	8.3	
Kings Mt., N. C., Appling sandy loam..	0-8	0.044	0.93	21.1	14.7
	8-18	0.027	0.34	12.6	
	18-36	0.018	0.19	10.5	
Cozad, Neb., Hall very fine sandy loam.	0-10	0.141	1.66	11.7	9.8
	10-18	0.105	1.13	10.7	
	18-24	0.072	0.66	9.1	
	24-36	0.047	0.37	7.8	
Grand Island, Neb., Cass fine sandy loam.	0-10	0.148	1.47	9.9	8.0
	10-18	0.069	0.65	9.4	
	18-24	0.024	0.19	7.8	
	24-36	0.021	0.11	5.2	
Rocky Ford, Colo., Las Animas clay.....	0-8	0.103	0.85	8.2	7.6
	8-18	0.071	0.58	8.1	
	18-24	0.043	0.31	7.2	
	24-36	0.044	0.32	7.2	
Lamar, Colo., Manville silt loam, Center Farm 1923.....	0-8	0.109	0.97	8.8	8.8
	8-18	0.096	0.90	9.3	
	18-36	0.037	0.31	8.3	
Lamar, Colo., Manville silt loam, Field No. 2, Center Farm 1924.....	0-10	0.118	1.06	8.9	

TABLE 1—*Continued*

LOCATION AND SOIL TYPE	DEPTH	NITROGEN	ORGANIC CARBON	RATIO C TO N-1	AVERAGE C TO N-1
	<i>inches</i>	<i>per cent</i>	<i>per cent</i>		
Rocky Ford, Colo., Rocky Ford Loam, Test plots 1924.....	0-13	0.095	0.90	9.4	
Rocky Ford, Colo., Rocky Ford fine sandy loam, Research plots 1925.....	0-8	0.128	1.20	9.3	7.2
	8-15	0.105	0.78	7.4	
	15-36	0.049	0.25	5.1	
Avondale, Colo., Rocky Ford fine sandy loam.....	0-8	0.107	0.88	8.2	7.3
	8-13	0.078	0.62	7.9	
	13-24	0.048	0.34	7.0	
	24-36	0.033	0.21	6.3	
Lamar, Colo., Otero sandy loam.....	0-10	0.101	1.00	9.9	
Alamosa, Colo., San Luis sandy loam....	0-10	0.074	0.56	7.5	5.6
	10-24	0.052	0.28	5.3	
	24-36	0.033	0.14	4.2	
Las Animas, Colo., Ft. Lyon Clay loam. . .	0-9	0.174	1.63	9.4	
Lamar, Colo., Prowers clay.....	0-8	0.140	1.28	9.1	7.8
	8-18	0.110	0.87	7.9	
	18-24	0.071	0.51	7.1	
	24-36	0.050	0.36	7.2	
Lamar, Colo., Prowers loam.....	0-9	0.140	1.31	9.3	
Lamar, Colo., Prowers loam	0-9	0.136	1.16	8.5	7.3
	9-18	0.082	0.62	7.5	
	18-36	0.051	0.31	6.0	
Lamar, Colo., Prowers clay loam	0-9	0.136	1.12	8.2	
McClave, Colo., Prowers clay loam.....	0-9	0.160	1.28	8.0	7.1
	9-20	0.088	0.66	7.5	
	20-36	0.051	0.32	6.2	
Wiley, Colo., Prowers clay loam, 1922 field.	0-8	0.150	1.36	9.0	7.2
	8-18	0.080	0.61	7.6	
	18-24	0.052	0.28	5.3	
	24-36	0.042	0.30	7.1	
Wiley, Colo., Prowers clay loam, 1923 field.....	0-8	0.210	2.01	9.5	7.2
	8-18	0.091	0.76	8.3	
	18-36	0.082	0.32	3.9	
Wiley, Colo., Prowers clay loam, 1924 field.....	0-9	0.174	1.63	9.3	

TABLE 1—*Concluded*

LOCATION AND SOIL TYPE	DEPTH	NITROGEN	ORGANIC CARBON	RATIO C TO N-1	AVERAGE C TO N-1
	<i>inches</i>	<i>per cent</i>	<i>per cent</i>		
Salt Lake City, Utah, Jordan sandy loam.....	0-10	0.134	1.12	8.3	6.8
	10-22	0.066	0.43	6.5	
	22-36	0.052	0.30	5.7	
Franklin, La., Collins silt loam, 1923 Experiment.....	0-10	0.154	1.74	11.2	8.1
	10-18	0.071	0.56	7.8	
	18-36	0.051	0.28	5.4	
Franklin, La., Collins silt loam, 1925 Experiment.....	0-10	0.135	1.26	9.3	6.9
	10-18	0.070	0.43	6.1	
	18-36	0.128	0.71	5.5	
Franklin La., Iberia silty clay loam, 1924 Irrigation Experiment.....	0-9	0.113	1.56	13.8	10.9
	9-18	0.066	0.65	9.8	
	18-36	0.065	0.61	9.2	
Franklin, La., Iberia silty clay loam, 1924 Experiment.....	0-10	0.125	1.41	11.2	18.3
	10-18	0.078	0.67	8.5	
	18-36	0.048	1.69	35.2	
Franklin, La., Iberia silty clay loam, 1925 Experiment.....	0-10	0.154	2.01	13.0	10.5
	10-18	0.125	1.53	12.3	
	18-36	0.073	0.46	6.3	
Monticello, Fla., Norfolk sandy loam.....	0-8	0.041	1.00	24.3	17.3
	8-18	0.031	0.58	18.7	
	18-36	0.033	0.30	9.1	
Cairo, Ga., Norfolk fine sandy loam.....	0-10	0.056	0.84	15.0	13.5
	10-18	0.026	0.40	15.3	
	18-36	0.029	0.30	10.3	
Pecan City, Ga., Greenville sandy loam.....	0-10	0.036	0.55	15.2	11.2
	10-15	0.026	0.31	11.1	
	15-36	0.023	0.17	7.4	
DeWitt, Ga., Greenville fine sandy loam.....	0-8	0.032	0.59	18.4	13.5
	8-15	0.032	0.44	13.7	
	15-36	0.026	0.22	8.4	
Baldwin, Fla., Bladen fine sandy loam.....	0-6	0.085	1.00	11.7	7.6
	24-36	0.045	0.16	3.5	

N varies from 0.01 to 0.08 per cent and C from 0.11 to 0.79 per cent. The C:N ratio varied from 11.5:1 to 1.8:1, the latter in a soil at the 36-inch level containing 0.06 per cent N and 0.11 per cent C. The water extract of this soil gave no reaction for ammonia, nitrates, or nitrites.

In considering the organic content of soils in relation to soil fertility, it is important to know, not so much the quantity of organic matter, but how much of it is available for supplying food for microorganisms. Since we know that the ratio of carbon to nitrogen in fresh vegetable material is 25:1 or more, and since such material is a good source of food for the microflora of the soil, it would seem that a C:N ratio approaching 20:1 might be considered as indicative of a fair supply of decomposable organic matter and that where the ratio is 10:1 or less, the organic matter is well advanced to the stage where further decomposition will be slow.

This conclusion, however, must be accepted with some reservations. The analytical figures in table 1 show that occasionally there is a high ratio at a low level (36 inches) where this could not be explained by recent additions of fresh organic material, but rather it would appear that such high carbon ratio might be due to an accumulation of organic compounds, the nature of which we, at present, know practically nothing. An inspection of the data, however, shows that abnormally high C:N ratios at lower levels are of rare occurrence.

In considering the relation of the C:N ratio to soil fertility, the ratio alone does not indicate the quantity of decomposable organic matter, since it is possible to have a C:N ratio much greater than 10:1 in the presence of but very small quantities of organic matter. For instance, in the Wickham fine sandy loam, at Fayetteville, North Carolina, with 0.34 per cent C, and a C:N ratio of 16.2:1, the total organic matter calculated by the Van Bemmelen factor is only 0.5 per cent; whereas the Davidson clay loam, from Linwood, North Carolina, with 1.12 per cent C and a ratio of 16.8:1, contains on the same basis three times as much organic matter, viz. 1.64 per cent.

It is evident then, that although a high C:N ratio in a surface soil may be taken as an indication of the presence of organic matter that can be readily decomposed and thereby furnish food for microorganisms, it is no indication of the quantity present.

The calculation of total organic matter in soils is frequently made by multiplying the quantity of carbon dioxide obtained by combustion by the Van Bemmelen factor 0.471 (or C multiplied by 1.724). In view of the fact that soil organic matter is made up of numerous compounds with different carbon content, such calculation can be considered but an approximation and is so regarded where it is intelligently used. It has been proposed to calculate the organic matter by multiplying the nitrogen content by a factor—20 being proposed. In view of the fact that soils contain organic compounds that are nitrogen free and that the nitrogen in those containing nitrogen is variable, the use of such a factor could again be nothing more than an approximation, with the advantage, if any, of eliminating the carbon determination.

A few calculations made from the data in table 1 disclose the fact that there is only occasionally an agreement in the organic content calculated by these two methods. For example:

Iberia silty clay loam Franklin, La.	0-10	$N \times 20 = 3.08$ organic matter $C \times 1.724 = 3.45$ organic matter
Hall fine sandy loam	0-10	$N \times 20 = 2.82$ organic matter $C \times 1.724 = 2.86$ organic matter
Prowers loam Lamar, Colo.	0-9	$N \times 20 = 2.80$ organic matter $C \times 1.724 = 2.25$ organic matter
Prowers clay loam Wiley, Colo.	0-8	$N \times 20 = 4.20$ organic matter $C \times 1.724 = 3.45$ organic matter
Portsmouth sandy loam New Bern, S. C.	0-12	$N \times 20 = 1.04$ organic matter $C \times 1.724 = 1.65$ organic matter
Davidson clay loam Linwood, N. C.	0-7	$N \times 20 = 1.32$ organic matter $C \times 1.724 = 1.93$ organic matter
Caribou loam Presque Isle, Me.	0-8	$N \times 20 = 4.54$ organic matter $C \times 1.724 = 5.11$ organic matter
Sassafras sandy loam Bridgetown, Va.	0-8	$N \times 20 = 1.04$ organic matter $C \times 1.724 = 0.87$ organic matter

Although the figures for Hall fine sandy loam and Sassafras sandy loam are in close agreement, in the others the differences are from 10 to 50 per cent of the total. Since the Van Bemmelen factor is based on a large volume of analytical work and is almost universally recognized as giving an approximation, the calculation of organic matter by multiplying the N by the factor 20 does not appear to have much to recommend it. This is further emphasized by calculating the organic matter $N \times 20$ and then calculating the percentage of carbon in the organic matter from the total organic carbon in the soil.

Portsmouth sandy loam New Bern, S. C.	0-8	$N \times 20 = 1.04$ organic matter Total C = 0.96 which gives organic matter containing 92 per cent C.
Marlboro sandy loam Lucama, N. C.	0-8	$N \times 20 = 1.10$ organic matter Total C = 0.99 which gives organic matter containing 90 per cent C.
Norfolk fine sandy loam Portsmouth, Va.	20-36	$N \times 20 = 0.46$ organic matter Total C = 0.10 which gives organic matter containing 22 per cent C.
Portsmouth sandy loam New Bern, S. C.	12-36	$N \times 20 = 0.56$ organic matter Total C = 0.68 which gives organic matter containing 121 per cent C.

Except for particles of coal, graphite, or charcoal, it is not at all likely that there are organic compounds in soils containing 90 to 92 per cent carbon, and the occurrence of any containing as little as 24 per cent carbon is also unlikely. The figure 121 per cent Portsmouth sandy loam indicate the absurdity of this calculation.

SUMMARY

Analytical data obtained from 176 samples of soil from 63 locations in 12 states show that the C:N ratio is quite variable; and, with a few exceptions, is highest in surface soil and becomes less at lower levels.

It is suggested that some idea of the availability of the organic matter as food for microorganisms may be indicated by the C:N ratio in surface soils.

The data show the futility of attempting to calculate the organic matter by multiplying the total N by the factor 20, as has been proposed.

THE INDIRECT DETERMINATION OF VARIOUS SOIL CHARACTERISTICS BY THE HYDROMETER METHOD¹

GEORGE JOHN BOUYOUCOS

Michigan Agricultural Experiment Station

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In former communications the hydrometer method has been suggested as a rapid, simple, and reasonably accurate method for determining the total colloidal material in soils in only 15 minutes (3); for determining the total amount of combined sands, silt, and clay or colloids in only 15 minutes (4); and for making a very detailed mechanical analysis of soils (5).

Work is being continued on this method with the idea of improving it, if possible, and also of trying to check it with other methods.

One of the phases of soil physics which has lately been investigated by the hydrometer method consists of ascertaining the relationship that exists between the soil material which the method determines at the end of 15 minutes and designates as the "total colloidal content of soils," and the physical properties or characteristics of soils, such as moisture equivalent, unfree water, and heat of wetting ratio.

EXPERIMENTAL

The relationship that the colloidal material, as determined by the hydrometer method at the end of 15 minutes, has to physical characteristics of soil, has been investigated thus far on three different soil characteristics; namely, heat of wetting ratio (2), moisture-equivalent, and unfree water. Experimental data have already been presented on the first two (3, 6) as well as on their indirect determination. In the present paper the results of a study on the unfree water, and additional data on the moisture equivalent will be presented.

Methods

The procedure consisted of ascertaining first, by the hydrometer method (3), the total colloidal content of the soils to be used in the investigation. Then their moisture equivalent and unfree water content were determined by the new moisture equivalent (6) and dilatometer methods (1) developed in this laboratory. The dilatometer method was somewhat modified in the present work. It consisted of placing 20 gm. of soil, based on oven-dry basis, into the

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special 50-cc. dilatometer, adding 10 cc. of water in the case of sandy soils and 15 cc. in the case of clay soils, and stirring the mixture thoroughly by means of a wire. The dilatometer was then filled half full with ligroin of very high boiling point, connected to a bicycle pump and suctioned carefully to eliminate any air in the soil. The dilatometer was then entirely filled with ligroin; stoppered with a cork stopper, the top of which was smeared with tallow; and then placed into an ice bath having a temperature of 0.5°C . The dilatometer was left in this bath, with frequent stirring, until it had completely attained the temperature of the bath and then the reading of the ligroin column on the dilatometer stem was recorded. The dilatometer was then taken out and placed into another ice bath having a temperature of about -10°C .; the reason for transferring from one temperature bath into another, is that at -0.5°C ., it is difficult to start solidification of the soil water and it requires a long time for complete solidification to take place, but around -10°C ., solidification is rapid, complete, and certain. After the soil water was completely frozen, the dilatometer was transferred again to the original temperature bath of -0.5°C . and allowed to remain there until it attained this original temperature. The ligroin column was again read on the dilatometer stem and from the difference of the two readings the amount of water that failed to freeze at -0.5°C . was calculated. The calculations were based upon the experimental finding by this dilatometer method that 1 cc. of water at 0°C . expands 0.1 cc. upon freezing.

In order that the dilatometer results of the various soils might be comparable, all the soils were washed several times with distilled water for the purpose of eliminating their soluble salts.

Data

In table 1 are shown the amounts of water that failed to freeze at -0.5°C . for a group of representative soils, also their colloidal content and moisture equivalent, and the relationship or ratio that exists between any two of them.

The data in table 1 show that the percentages of colloids, unfree water, and moisture equivalent vary widely for the different soils, all three tending to be high with fine-textured soils and low with coarse-textured soils. When these results are reduced to a ratio basis by dividing the unfree water by the colloids, the moisture equivalent by the unfree water, and the moisture equivalent by the colloids, their significance is revealed. Then it is at once seen that all the different soils have a close relationship within each of the three different ratios; and when it is considered that these different soils vary greatly in their chemical composition and in some of their physical characteristics, these ratios are remarkably close. It will be seen that most of the soils in all three cases give a ratio close to the average. The soils the ratios of which seem to vary the most from the averages, are those containing organic matter, as exemplified by Fargo clay loam, Minnesota Clyde silt loam, and Michigan silt loam. One reason that organic soils give a higher ratio is probably because some of the organic matter is almost impossible to disperse and consequently the hydrometer

method may not determine the entire colloidal content (3). Also, since organic matter has a lower specific gravity, and the hydrometer has been calibrated on average loam soil suspension, a smaller amount of suspended material will be shown than there actually is.

At this juncture, it must be also stated that some abnormal soils do not give as close ratios as the ordinary or normal soils shown in table 1. By "abnormal soils" is meant calcareous soils that may contain 50 per cent or more of carbonates; certain subsoils such as the Susquehanna clay C horizon, which seem to be composed mostly of Fuller's earth; mucks and peats; and undecomposed

TABLE 1

Total soil colloids, unfree water, and moisture equivalent and the relationship that exists between any two of them in the various types of soil

SOIL	COL- LOIDS	UNFREE WATER	MOIS- TURE EQUIV- ALENT	RATIO U W * COLLOIDS	RATIO M E † U W	RATIO M E COLLOIDS
	per cent	per cent	per cent			
Ontario silt loam B	65.0	16.5	38.00	0.254	2.303	0.5843
Fargo clay	62.0	20.0	49.00	0.322	2.450	0.7900‡
Cecil clay loam B	60.7	15.9	38.40	0.262	2.415	0.6328
Ontario silt loam A	58.5	15.2	36.50	0.257	2.402	0.6240
Miami silt loam	54.5	13.6	32.10	0.250	2.360	0.5890
(Ia.) Bremer clay	53.6	13.5	33.20	0.252	2.460	0.6194
Napanee clay loam A	47.4	12.4	31.10	0.262	2.508	0.6560
(Minn.) Clyde silt loam	43.0	12.2	33.20	0.284	2.721	0.7720‡
Manor schist loam B	32.5	9.3	19.80	0.286	2.130	0.6093
Brookston clay loam A ₁	30.0	8.9	18.60	0.297	2.090	0.6200
(Mich.) Silt loam	26.0	7.6	15.50	0.292	2.040	0.5960
(Mich.) Silt loam	22.4	7.2	15.90	0.321	2.209	0.7100‡
Fox loam B ₁	21.4	7.1	14.85	0.322	2.092	0.6940
Strong's sandy loam	10.4	3.2	6.50	0.308	2.032	0.6250
Average				0.2835	2.301	0.6515

* U. W. = unfree water.

† M. E. = moisture equivalent.

‡ Soils containing high content of organic matter.

organic matter in general. For such abnormal soils, probably the best method to use for determining their total colloidal content is the indirect method of the moisture equivalent (6). By determining the moisture equivalent of these soils by the procedure already described their total colloidal content can be ascertained by dividing the percentage of moisture equivalent by the average ratio of moisture equivalent.

When this procedure is applied to Fargo clay and Clyde silt loam in table 1, it is found that the colloidal content of these soils is considerably increased, and their ratio is thereby brought much closer to that of the inorganic soils.

It must be remembered, however, that the difference in the specific gravity between the inorganic and organic soils, introduces many complexities which must be taken into consideration in any comparative relationship studies.

Aside from these abnormal or special cases, the results in table 1 tend to show quite conclusively that the material which the hydrometer method determines at the end of 15 minutes and designates as colloids, practically controls the physical characteristics of soils, at least those that deal with moisture relationships. If this were not the case, the ratios would not be so close for the various types of soil.

These ratios reveal one more significant thing, namely, the hydrometer for determining colloids, the dilatometer for determining unfree water, and the vacuum pressure method for determining moisture equivalent of soils, would seem to be fundamentally sound and correct, at least they tend to give true comparative results for the various soils.

Indirect determinations

Since the relationships between colloids and unfree water, between unfree water and moisture equivalent, and between colloids and moisture equivalent are so close, when one result is known the other can be calculated from the respective average, as indicated by the formulas:

$$\text{Colloids} \times \frac{\text{Moisture equivalent}}{\text{Colloids}} \text{ ratio} = \text{per cent moisture equivalent,}$$

$$\frac{\text{Moisture equivalent}}{\text{Moisture equivalent}} \frac{\text{Colloids}}{\text{Colloids}} \text{ ratio} = \text{per cent colloids,}$$

$$\text{Colloids} \times \frac{\text{Unfree water}}{\text{Colloids}} \text{ ratio} = \text{per cent unfree water,}$$

$$\frac{\text{Unfree water}}{\text{Unfree water}} \frac{\text{Colloids}}{\text{Colloids}} \text{ ratio} = \text{per cent colloids,}$$

$$\frac{\text{Moisture equivalent}}{\text{Moisture equivalent}} \frac{\text{Unfree water}}{\text{Unfree water}} \text{ ratio} = \text{per cent unfree water.}$$

Wilting coefficient

The unfree water shown in table 1, has a special interest and significance in that it might represent the wilting coefficient of plants, and in that event, the latter can be determined indirectly very rapidly and simply by the hydrometer method.

The belief that the unfree water of the various soils as obtained by supercooling and freezing them at the temperature of -0.5°C . might represent their respective wilting coefficient points, is based upon two well-founded facts: First, the careful researches of Shull (8) on the measurement of the surface forces of soils, seem to show that the suction force of the soil for water at the wilting point is about 4 atmospheres; second, according to the laws of the freezing point depression the suction force of soil for water at the freezing point depression of -0.5°C . is about 6 atmospheres. This latter result is obtained from the well-known facts that a molar solution has an osmotic pressure of about 22.4 atmospheres and that the freezing point depression of a water solution containing 1 gram molecule per liter of non-ionized salt is 1.86°C . According to this figure, and assuming the relationship is linear, the suction forces of soils for water corresponding to various freezing point depressions are about 5.95 atmospheres for -0.5°C ., 17.9 atmospheres for -1.5° , 29.8 atmospheres for -2.5° , 47.7 atmospheres for -4° , and 937 atmospheres for -78° .

If the unfree water then as obtained at the temperature of -0.5°C . does actually represent the wilting coefficient points of soils, then the wilting coefficient of practically any soil can be determined very simply in only 15 minutes by means of the hydrometer method. The formula that may be used to accomplish this indirectly is as follows:

$$\text{Percentage colloids} \times 0.2835 = \text{wilting coefficient},$$

Since the results obtained by the foregoing formulas have not been checked as yet by actual experimentation with plants, it cannot be said how correct they are. Judging, however, from the apparent soundness of the principles on which they are based, and also from a comparison of magnitude of actual wilting coefficient data, as obtained by Briggs and Shantz (7) on somewhat similar soils, it would appear that they might be quite correct, at least in the normal soils.

It is thus seen then, that on account of the great rapidity, distinct simplicity of manipulation, and reasonable degree of accuracy, the hydrometer method may not only be employed to estimate the colloidal content of soils very rapidly, but may also be used to determine very quickly several characteristics of soils. These indirect determinations, however, should be regarded only as approximately correct.

SUMMARY

Experimental results are presented which tend to show that the soil material which the hydrometer method determines at the end of 15 minutes and designates as total colloids of the soil, has a close relationship to the moisture equivalent and unfree water of soils.

On account of this close relationship, these physical characteristics of soils may be indirectly determined very quickly by the hydrometer method.

It also appears that the wilting coefficient of soils also may be indirectly determined by the hydrometer method very rapidly.

These various indirect determinations should be regarded only as approximately correct.

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LOCAL VARIATION OF SOIL ACIDITY IN RELATION TO SOYBEAN INOCULATION

GEORGE Z. DOOLAS¹

University of Missouri

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Irrespective of the extensive investigation on the injurious effects of soil acidity on inoculation and growth of legumes, the nature of these effects is not yet fully understood. The question naturally arises: is the failure of nodulation due to the detrimental effects of the hydrogen-ion or hydroxyl-ion concentration directly on the legume bacteria or does the concentration in some way bring about unfavorable conditions in the plant which prevent the entrance of the specific bacterium or prevent nodule formation even if the bacterium is present in the plant tissue. Since this question has an important bearing on the problem of obtaining good legume inoculation in certain acidic soils, the experiments reported herein were conducted to determine whether successful inoculation might be obtained by reducing the acidity in only a portion of the soil; and in the meantime to study the effects of different hydrogen-ion concentrations in scattered local areas in the same soil on nodulation, root growth, and top growth of soybeans.

LITERATURE REVIEW

Fred and Davenport (7) found that the favorable reaction range of *Bac. radicola* is closely correlated with that for its respective host. As a consequence of their findings, they were enabled to classify *Bac. radicola* from different plants according to pH limits for growth or reproduction. For the soybean organism the critical acidity was found to be at pH 3.3. Bryan (4) observed that the limits for inoculation of soybeans were pH 4.6 and 8.0, whereas those for the growth of the soybean plant were pH 3.8 and 9.6. The aforementioned data suggest that if the lack of nodulation is ascribable to some failure of the organism, certainly it is not because the organism is not present, since it will live in a hydrogen-ion concentration (pH 3.3) far beyond the limits (pH 4.6) in which it will inoculate. However, it is possible that it loses its power of motility or virulence to enter into the plant root tissues, or if it enters, it may fail to perform its normal function.

Snieszko (17) more recently found that either too high or too low pH induced the production of the vacuolated form of legume bacteria, which is thought by Bewley and Hutchison (3) and by Thornton and Gangulee (18) to lack the power to inoculate. According to Thornton and Gangulee, milk and calcium phosphate in the inoculum suspensions favor the production

¹ Thanks are due to Dr. Wm. A. Albrecht, under whom the author worked, for his valuable criticisms and suggestions in connection with the present work and the preparation of this paper.

of the supposedly virulent forms; and on this basis they recommend the inoculation of the soil with bacterial suspension in milk to which is added 0.1 per cent of calcium phosphate. They attribute the beneficial effects to the phosphorus.

Wilson (21) Perkins (14), and Kellerman and Robinson (10), have found the calcium compounds, with the possible exception of the sulfate and nitrate, effective in stimulating nodule formation in sand and soil cultures under greenhouse conditions.

Scanlan (16) and Albrecht and Davis (1) obtained increases both in nodulation and growth of legumes on several Missouri acid, and supposedly calcium-deficient, soils to which either basic or neutral salts have been added both under greenhouse and field conditions.

Bryan (4) observed that outside of certain limits of the hydrogen-ion concentration of the growing medium in which nodulation was depressed, the roots were discolored and injured, whereas the secondary roots were stubby.

Whitson and Chapman (20) report that in many soil types in Wisconsin, acidity is not the only factor limiting growth but that deficiency of calcium, of potassium, or of phosphorus may also be a factor.

Since acidity in soils is generally associated with low amounts of calcium, and since legumes utilize comparatively large amounts of this element, it is difficult to ascertain whether the limiting factor is the reaction or the deficiency of calcium. True (19) has pointed out that calcium is the most important single element, especially during the early stage of plant growth, since it plays a unique rôle in the middle lamellae of the plant and in absorbing mechanism of the plant root. He found that the magnitude of the limiting quantity of calcium and its absorbability from dilute concentrations of calcium in solution are very closely correlated with the degree of acidity tolerance of plants. The legumes were found to be among those plants which normally require comparatively large amounts of calcium for normal growth to maturity.

Arrhenius (2) carried out extensive researches on the influence of the different forms of acidity, such as free, exchangeable, and hydrolytic, on plant growth and on the absorption of the nutrients. Mevius (13) quotes some data from Arrhenius on the absorption of the nutrients at different pH values, and states that the absorption is proportional to the pH; however, the permeability of the cell does not depend on the H or OH ion alone, but also on the kind, number, and mutual relationships of the other ions. Bryan (5) observed a similar decrease of calcium content in alfalfa, alsike clover, and red clover with an increase of acidity of the water cultures in which they were grown. Kirste (11) reports that the unfavorable influence of the exchangeable acidity could be removed by treatment of some of the more susceptible crops such as barley, beets, and mustard, not only with calcium carbonate but also with soda.

Jansen (8), working with some southern legumes, obtained increased growth and nodulation by modifying the acidity by addition of sodium hydroxide as well as of calcium hydroxide. Karraker (9) grew alfalfa in pots with part of the roots in limed and part in unlimed acid soils and concluded from the results obtained that the effect of soil reaction upon nodule formation must be one of localized character in the plant, or a direct effect of soil hydrogen-ion concentration on the bacteria. Albrecht and Davis (1) grew soybeans with a part of the roots grown in an unlimed portion and part in a limed portion of an acid soil. From the results of this and other experiments, they concluded that calcium is beneficial and that its effect on nodulation is local in character.

McCool (12) observed that local application of small amounts of lime drilled with the seed resulted in very good increases of alfalfa crop in many acid soils in Michigan.

The studies mentioned in the review reveal two interesting points. The first suggests that other factors, particularly calcium deficiency, may influence the effects of soil acidity on inoculation. The second leaves the impression that soil acidity, and calcium as well, may affect legume inoculation through (a) their influences on the plant and (b) their influences on the bacteria. For fuller knowledge of how soil acidity injures inoculation it is necessary, therefore, (a) that the calcium deficiency factor be eliminated, and (b) that a differentiation

be made between the plant and the organism, as each may be influenced by acidity in respect to inoculation. Accordingly, in the following experiments whereby it is intended to study the effect of local variation of hydrogen-ion concentration on inoculation, an attempt was made to eliminate the factor of calcium deficiency, and thus attempt to measure only the effects of the hydrogen-ion concentration on inoculation as they may be wrought through the plant and the organism separately.

EXPERIMENTAL

The soil used in these experiments was surface Putnam silt loam from a field in which inoculation of soybeans was observed to be difficult. It is of medium fertility and has a reaction of pH 4.0 to 5.5. Its electro dialyzable calcium content is only 4 m. e. to 100 gm. of soil. The titration curves, as

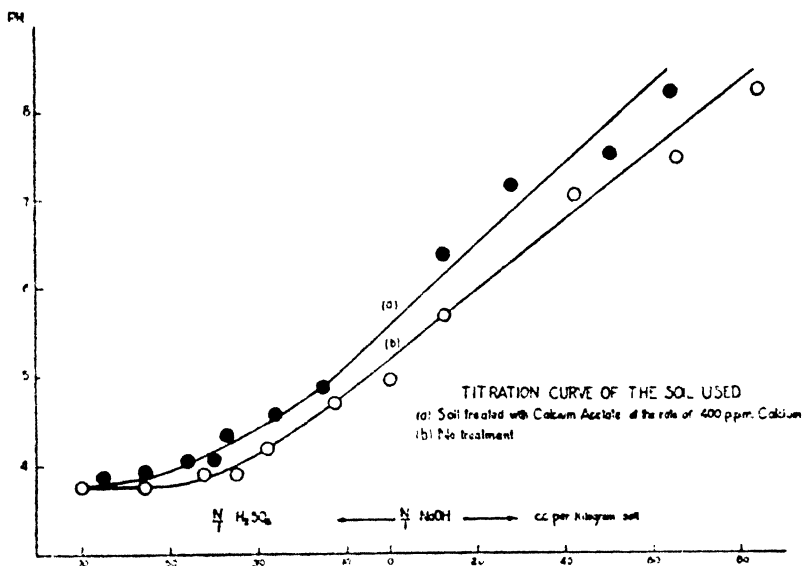


FIG. 1. TITRATION CURVE OF THE SOIL USED

determined from the reaction changes after equilibrium had been reached, are presented in figure 1. When calcium acetate was added a larger quantity of acid and a smaller amount of base were required to obtain the same pH than when no calcium acetate was added.

Soybeans were grown under greenhouse conditions in four series of pots, designated as A, B, C, and D. Series A and B were prepared specially for the study of the effects of local variation of the hydrogen-ion concentration. An attempt was made in these to separate the influences of the hydrogen-ion concentration on inoculation as they may be effected (a) through the plant and (b) through the organism. This was deemed possible by growing the roots of the plant through different soil areas varying in reaction. In these pots the soil was divided into two zones by means of paraffin screen pots. These

pots were made by dipping pot-shaped, screen wire frames into a molten mixture of 4 parts paraffin and 1 part petrolatum. This material when solidified, allows the penetration of the roots without permitting any leakage at the points of root penetration. Into each of these paraffin pots 400 gm. of soil was placed. They were buried, almost to their tops, in 2,500 gm. of soil contained in gallon jars. After the treatments and after the air-dried soil was mixed in the ball mill, the pots with moistened soil were allowed to stand for about 30 days in order that an equilibrium might be reached. The soil both in the paraffin pots and in the jars was inoculated.

Presumably, therefore, this system contained both the bacteria and the plant roots in two separate zones which differed in reaction and calcium content. Such conditions permitted the plant at least to draw its nutrients from the zone of favorable reaction. If acidity is only local in its effect on inoculation, nodules would be formed, at least under these conditions, on the roots in the zone of the favorable reaction. Conversely if it is general, or systemic, through its effects on the plant, then irrespective of the presence of the favorable soil environment for the bacteria no nodules would be likely to form in any part of the roots.

Series D was prepared in order to ascertain whether the calcium as supplied by the calcium acetate additions would modify to any perceptible extent the influence of acidity on inoculation. Series C and D, added as a means of control for series A and B, consisted of 1-gallon jars each containing 3,000 gm. of soil. The reaction in these was changed in the same order as in the series A and B. In addition, series C and D included pots in duplicate of pH 7.0, 7.3 to 7.5, and 8.2 to 8.35. These ranges were effected by the addition of a dilute solution of sodium hydroxide. To pots of series D, calcium acetate was added in an amount equivalent to 2 m. e. of calcium to 100 gm. of soil, corresponding to that added to series A and B.

Both the soil and seed were inoculated with artificial cultures. Five soybean plants were allowed to grow in each pot for 80 days. They were irrigated with distilled water, added on the surfaces in the case of the paraffin pots and through a small flower pot sunk in the soil.

RESULTS

Nodule formation was good and of about equal extent in all jars of series A, or in those soil zones (pH 5.6) outside of the paraffin pots, except in one case in which the reaction in the paraffin pot was initially brought to a pH as low as 3.8. In this case not only were nodules absent, both inside and outside of the paraffin pot, but even root growth was very poor, being badly injured within, and very sparse without, the paraffin pot. The failure of nodule formation exterior to this most acid inner zone appears to have been the result of nutritional disturbances brought about probably through injury to the inner roots. These results are evident from the data in column 4, series A, table 1, and are shown clearly in the upper halves of figure 1 and 2 of plate 1. As noted from

the nodule counts in column 5, series A, table 1, nodulation was absent inside the paraffin pots of initial acidities ranging from pH 3.8 to 4.6, but was present in those of initial acidities of pH 4.6 and pH 5.0

TABLE 1
Effect of local variation in soil reaction on the nodulation and growth of soybeans
(Series A and B)

SULFURIC ACID ADDED*	pH OF SOIL		NODULES PER POT (AVERAGE)		WEIGHT PER POT (AVERAGE)		CONDITION OF GROWTH
	Plant- ing	Har- vest					
cc							
Inside†	Inside	Inside	Out- side	Inside	Tops	Roots	
Series A							
0	5.05	5.15	10	20	1.75	0.38	Roots healthy, inside and outside
0.72	4.65	5.0	6	7	1.65	0.31	
1.75	4.35	4.6	9	0	1.64	0.32	Out, good; inside, brown, acid-injured
3.20	4.175	4.2	12	0	1.70	0.27	Out, fairly good. Inside, no secondary roots
3.57	3.9	4.1	20	0	1.50	0.38	Primary, acid-injured
4.7	3.8	4.0	0	0	1.15	0.15	Out, few roots. Inside badly injured
Series B							
Outside‡	Out- side	Out- side	Inside	Out- side	Tops	Roots	
0	5.0	5.0	37	0	1.87	0.35	Good, inside and outside
0.72	4.6	4.85	23	0.2	2.09	0.29	
1.75	4.3	4.6	34	0	1.90	0.40	Inside good. Out, few secondary roots, all black and acid-injured
3.20	4.0	4.2	32	0	1.34	0.33	
3.50	3.95	4.0	30	0	1.36	0.30	Inside, good. Out, few or none
4.50	3.875	4.0	1-29	0	1.76	0.39	Inside, good. Out, none

* Normal sulfuric acid per 100 gm. soil.

† Term "inside" refers to soil within the screen paraffin pot given the sulfuric acid. The soil outside of the screen pot received calcium acetate equivalent to calcium at 400 p.p.m. of soil, giving it a pH of 5.6 to 5.8 constant throughout the growth period.

‡ The term "outside" refers to the soil exterior to the screen paraffin pot. Soil treatments were the same as* but reversed with reference to location inside or outside the paraffin pot.

In the case of series B, in which the acid and more favorable reaction zones were arranged in a reverse order to that in series A, the effects on nodulation and root growth were also correspondingly reversed. Accordingly, nodules were absent in the jars or in the zones outside of the paraffin pots of pH 3.8 to 5.0 except in one of the duplicate pots of pH 4.6, but were abundant inside

of the paraffin pots or the zones of pH 5.6 (columns 4 and 5, series B, table 1 and lower halves of figures 1 and 2 of plate 1).

The data reveal a correlation between increase of acidity and increase of injury to the roots below pH 4.6. Beyond this limit they were black and rather tough, the primary roots were fewer, short, and stubby. In general, root growth decreased with increase of acidity within its zone.

TABLE 2

Nodulation and growth of soybeans when the entire root system grew in soil uniform in reaction
(Series C and D—spring planting)

POT TREATMENT	pH	NUMBERS PER POT		WEIGHT PER POT		CONDITIONS OF GROWTH
		Plants	Nodules	Tops	Roots	
				gm.	gm.	
*C	3.80	0	0, 0	0	0	Cotyledons appeared but no leaves were formed
†D		0	0, 0	0	0	
C	3.85-3.95	5	1, 0, 0, 0	1.11	0.13	Roots and tops were injured; leaves dried at margins and tips, and most of them died
D		5	7, 1, 0, 0	1.15	0.15	
C	4.10-4.25	5	0, 0, 0, 0	1.02	0.13	
D		5	2, 0, 0, 0	1.20	0.11	
C	4.35	5	0, 0	1.77	0.20	Tops injured. Roots black
D		5	0, 0	1.53	0.16	
C	4.65	5	0, 0	1.39	0.17	Tops and roots moderately injured
D		5	0, 0	1.25	0.13	
C	5.0	5	5, 3	1.91	0.22	Roots healthy. Tops, slightly injured
D		5	1, 0	1.30	0.15	
C	7.1-7.2	5	0, 13	2.00	0.12	Soil puddled and too wet. Tops good. Roots short but healthy
D		5	0, 16	3.10	0.16	
C	7.3-7.5	5	0, 0	0.50	0.06	
D		5	0, 1	1.30	0.06	
C	8.2-8.4	0				Soil too wet. Sparse root growth
D		3, 0	0, 0	0.475	0.07	

* The physical condition of the soil was poor in the non-calcium alkaline pots, but it was better in the calcium-treated pots.

† D—400 p.p.m. calcium added per pot.

As one might expect, the magnitude of disturbance of nodule formation was more or less parallel with the degree of root injury. Thus, nodule formation was either depressed or totally absent in the root segments that were damaged, whereas it was normal in the root segments that were healthy. However, the extent to which nodule formation took place on an apparently equally

damaged root segment appears to have varied according to some not well understood factors, probably such as nutrition disturbances that might have been caused through injury to other parts of the plant. Nodule formation, therefore, tended to vary, in general, according to the healthiness of the local root tissue and according to the nutritive supply to it.

There were greater numbers of nodules in the inner than in the outer zones of the same pH. A critical study of nodule counts given in column 5, table 1, suggest that the critical reaction limit for nodule formation occurred at a higher pH for the roots in the outer zone, or more distant portion of the roots, than for the roots closer to the base of the stem which were grown in the inner zone. Both nodule numbers and root growth decreased according to the decrease of pH in the soil area, but remained relatively constant in the zones of constant pH (5.6). Figure 1 of plate 1 and the data in columns 6 and 7, table 1, show that the depressing effects of acidity were less on the growth of tops, than upon the nodulation, and upon root growth.

No definite correlation was observed to exist between the amount of top growth and the relative abundance of nodules.

In both series C and D, in which the entire medium had the same reaction, nodule formation and growth of both tops and roots were markedly subnormal and decidedly inferior to those of the pots of the corresponding reaction in series A and B, as shown in plate 2 and table 2. Nodulation occurred rather irregularly. For example, few nodules were found on some plants grown in soil of reaction pH 4.0, whereas none was found in some pots of reaction higher than pH 4.0. It is interesting to note that nodules occurred very frequently in that portion of the roots lying just below the small pot through which irrigation water was applied. Incidentally this circumstance suggests the possibility that inoculation was influenced by the local environment as it might have affected the root segment directly.

Unlike series A and B, the tops in the pots of series C and D exhibited marked irregularities, which increased with decrease in pH. The leaves were curled, beginning first at the tips, which were burned and folded inward. They were dried at the margins but remained green toward the midrib.

There were no perceptible differences with respect to nodule formation and root and top growth between the pots with and without calcium additions, except in the alkaline ranges of 7.0 to 8.3 wherein calcium might have been beneficial. Calcium might have reduced the bad physical condition of the soil by counteracting the deflocculating effects of the added sodium hydroxide, or it might have been a source of soluble calcium to overcome the possible decreased solubility of the soil supply. Since calcium was apparently not a factor in inoculation or plant growth in this case, the influence of the hydrogen ion seems to be responsible for the nodulation irregularities.

The behavior of roots toward acidity was the same as those in the corresponding acid zones of the divided pots, series A and B. The character of the injury exhibited on roots was similar to that observed by Bryan (4). In many re-

spects the irregularities observed on the plants in general, and particularly on the tops, seems to have been very similar to those observed generally in soybeans grown in this greenhouse in calcium-deficient media.

The extent to which acidity inhibits or prohibits inoculation, appears, according to general observation in these trials, to be influenced to some extent

TABLE 3

Nodulation and growth of soybeans when the entire root system grew in soil of uniform reaction
(Series C and D—Summer Planting)

POT TREATMENT	pH	NODULES PER POT (AVERAGE)	WEIGHT PER POT		CONDITION OF GROWTH
			Tops	Roots	
			gm.	gm.	
C	3.75-4.00	0	2 00	0.25	Leaf and root injury increased with decrease in pH. Few short and stubby secondary roots
D*		0	1 50	0 15	
C	4 15-4 20	1	2 30	0 17	
D		2	1 82	0 18	
C	4.25-4.30	2½	2 2	0 24	Crown roots were brown or black; leaves were injured
D		0	1 9	0 18	
C	4 40-4 50	14	3 23	0 30	
D		2	2 90	0 19	
C	4.55-4.65	12	3 15	0 39	Roots and tops healthy but crowns were brown
D		7	2 50	0 19	
C	4 90-5 00	17	2 90	0 38	
D		4	2 55	0 34	
C	6 0	30	3 6	0 38	Both roots and tops healthy; leaves deeper green than in the above
D		20	3 0	0 30	
C†	7 0	22	3 7	0 25	
D		35	3 4	0 30	
C	8 3	4	3 0	0 20	
D		18	2 8	0 25	
D	8 70	0	2 30	0 18	

* D = 400 p.p.m. calcium added per pot.

† The soil in the alkaline pots was diluted with acid-extracted quartz sand.

by the weather factors, particularly light, which is difficult to control. Accordingly, the actual lower pH limit is not necessarily always a fixed value. When the pots of series C and D (table 3), were replanted in August, 6 months after the first planting, nodule formation was greater and growth of tops and roots was generally less injured; and nodulation took place at a lower pH,

than in the first planting, which took place in the winter and spring months. These observations suggest that the extent to which acidity becomes injurious to inoculation is also dependent on the external factors, which may influence the growth of the host plant.

It is evident from the reported observations that the harmful effects of acidity on nodulation and root growth were, in the pH ranges employed here, namely pH 4.0 to 5.6, more or less local in character. The extent to which acidity injured other parts of the plant outside of the acid zones, appears to have been determined by the extent to which the nutritive supply to those

TABLE 4
Inoculability of soybean organism from pots of varying reaction
(Test made in sterile sand culture pots)

SOURCE OF INOCULATING MATERIAL		AVERAGE NUMBER OF NODULES PER PLANT IN THE SAND CULTURE POTS
Pot	pH in the pot	
Commercial culture	6.0
A—inside.....	3.8	16.0
B—outside....	3.8	0.7
C.....	3.8	13.0
D.....	3.8	8.0
A—outside.....	5.6	16.5
C.....	4.0	12.0
D.....	4.0	19.0
A—inside.....	4.65	14.0
B—outside.....	4.65	12.0
A—inside.....	5.0	10.0
C.....	5.0	12.0
C.....	7.0	4.0
D.....	7.0	13.0

other parts was disturbed. The very sparse growth of roots in the outer soil area exterior to the most acidic inner zone (pH 3.8), and the abnormalities of the tops in the pots (pH 3.8 to 5.0) in series C and D, as compared with the absence of such abnormalities in the paraffin pots of series A and B, suggest such a case of nutrition disturbance.

Bac. radicola remains viable in acid soils

In order to ascertain more definitely whether depression or failure of nodule formation in these experiments was due to death or to loss of viability of the organism in these acid media, the following test was made. Four months

after the planting of the soybeans in the pots of the preceding experiments, a 2-gm. sample of soil was collected from each of the several pots representing different acid intensities. This sample was used to inoculate soybean seeds which had been planted in sand culture pots. Sterile control pots were planted with these inoculated seeds. The test was made with the utmost care in order to avoid contamination by soybean bacteria from other sources.

Table 4 reveals that in these sand cultures viable bacteria had been transferred from every single soil pot of the previous experiment, including those with the extreme acidity of pH 3.75, in which no nodules originally occurred. The nodule in these sand cultures were uniformly plentiful, save in one case of the sample of soil (pH 3.8) outside of the paraffin pot series B. The nodule numbers in this case were markedly lower than in the other pots. This apparent depression of either the viability or inoculability of *Bac. radicola* can not be attributed to the effects of strong acidity alone, for the reason that the viability of the organism was found to be just as good in the pots of the same pH of the other series. It must be remarked that these were the acid zones (pH 3.8) outside of the paraffin pots or jars, in which hardly any roots were grown. It may be possible, therefore, that the viability of the organism is favorably influenced by its association with the living roots of its respective plant host.

If the depression or prevention of nodule formation occurred because of the effects of acidity on the legume bacteria, such disturbances would result (a) if acidity prohibited entrance of the bacteria into the root, (b) if the reaction of the tissue disturbed the bacterial function of nodule production, or (c) if acidic conditions interfered with the nutritive supply to the organism and nodule tissue. Failure of the organism to enter the roots cannot be due to the death of the bacteria in the acid ranges used. Unfortunately no measurement of acidity of the root tissue was made. Since the roots were badly injured in the acid zones, the disturbance in the nutritive supply to the root area of nodule formation seems a plausible explanation.

SUMMARY

When soybeans were grown in the greenhouse with the roots extending partly through acid soil zones of pH 3.8 to 5.0 and pH 5.6, depression of nodulation and root injury occurred on the segments within the media with pH ranging from 3.8 to 4.6. In the soil area with pH 5.6 the root segments were healthy and, with but few exceptions, uniform in nodulation.

The paraffined screen pot permitted growing different portions of the same root in areas of different reactions within the same pot.

Apparently healthy plants of uniform growth resulted when part of the root system was partially injured in the extremely acidic soil areas (pH 4.0 to 5.6), and partially healthy plants in the less acidic media (pH 5.6). When the entire root system was surrounded by the extremely acidic soil, however, abnormalities in the growth both of roots and tops were evident.

It seems probable that depression of nodulation by acidity above the acid degree of pH 3.8 or 4.0 occurred through the action of the acidity on the plant tissue locally, or through the disturbance of the nutritive supply by the plant to the organism and tissue area in question.

Nodule organisms retained their viability in all soil media with degrees of acidity ranging from pH 3.8 to 8.3.

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PLATE 1

EFFECT OF VARYING DEGREES OF ACIDITY AS SOYBEAN GROWTH

FIG. 1. Plant growth and root development exterior to paraffin pots with soils of varying degrees of acidity.

Above: Reaction outside constant (pH 5.6); varied inside (pH 5.0 to 3.8).

Below: Reaction outside varied (pH 5.0 to 3.8); constant inside (pH 5.6).

FIG. 2. Root development of soybeans as affected by soils of different degrees of acidity within paraffin pots.

Above: Varying degrees of acidity in soil of plant crown area (pH 5.0 to 3.8) and constant acidity in remaining root zone (pH 5.6).

Below: Constant acidity in soil of plant crown area (pH 5.6) and varying degrees of acidity in remaining root area (pH 5.0 to 3.8).

GEORGE Z. DOOLAN

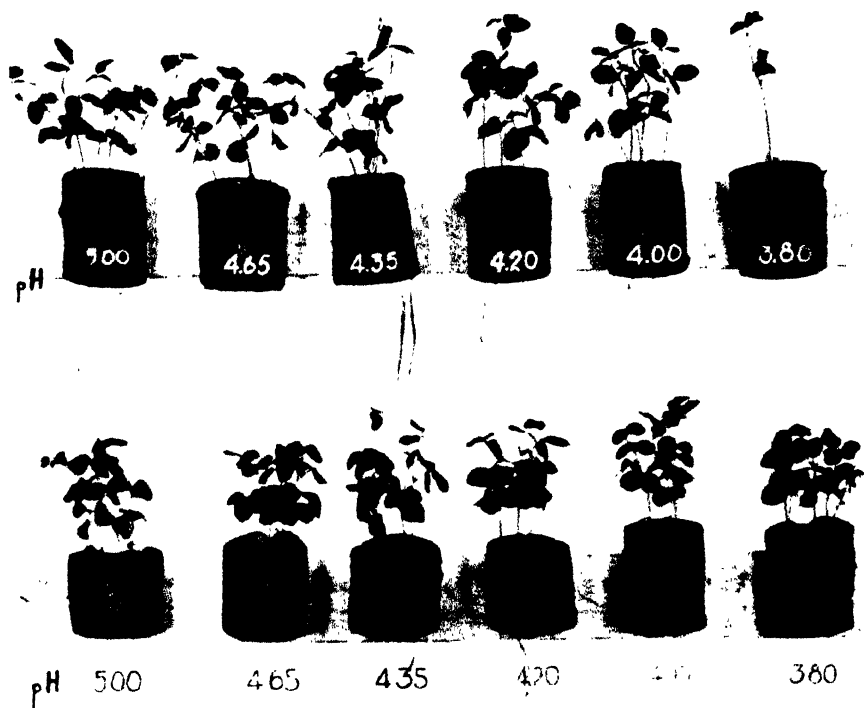


FIG. 1

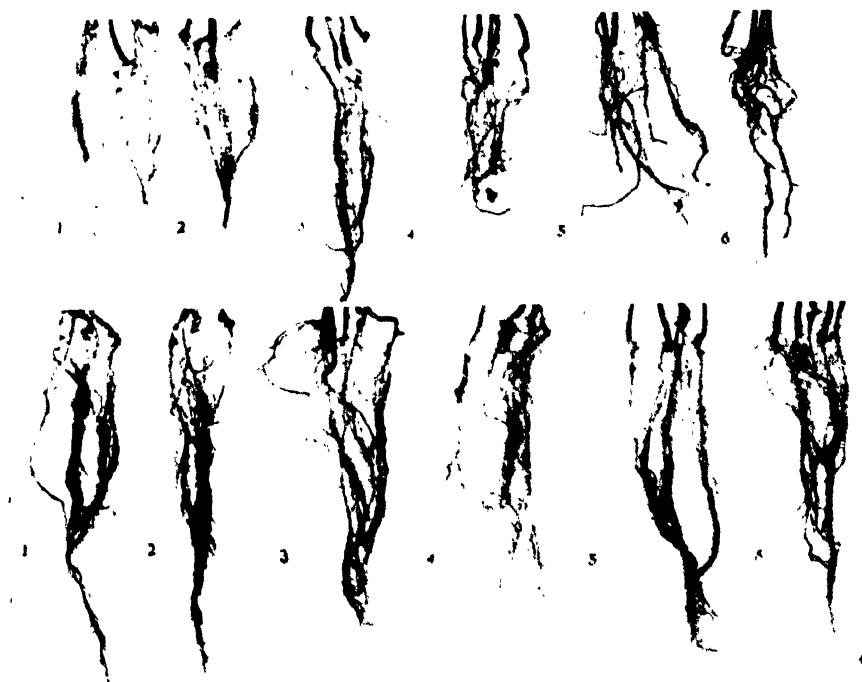


PLATE 2

SOYBEAN GROWTH IN CONSEQUENCE OF VARYING DEGREES OF ACIDITY IN THE SOIL

Above: Calcium acetate added, 400 p.p.m. Ca to each pot

Reaction: Left to right, pH 3.75, 3.90, 4.05, 4.20, 4.35, 4.60, 5.0, 7.4, 7.3, and 8.25



SEASONAL VARIATION IN THE NUMBER OF TWO SPECIES OF RHIZOBIUM IN SOIL

J. K. WILSON

Cornell University

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The growth and longevity of bacteria in soil are dependent upon climatic factors, the physical and chemical make-up of the soil, the composition of the soil air, the temperature, the time of year, the associative action of the edaphon contained in the soil. Since bacteria respond to their environment it would be expected that as any one or more of these factors vary the bacterial content would also change. Sometimes these factors operate to increase and sometimes to decrease the number of bacteria that may be found at any season. Studies have been made of the fluctuations which occur in bacterial number from one season of the year to another season, but they have been made of the heterogeneous flora that will develop on a certain type or types of media.

Francé (4) reports a minimum of the heterogeneous soil flora in winter with a maximum in spring and fall. Brown and Halverson (1) found from a similar study that during the summer and early fall the bacteria did not develop parallel with either moisture or temperature, and during much of the year other undetermined factors seemed to control bacterial development. It was found also that the soil bacteria decreased in late fall with a drop in temperature until the soil became frozen, when the number rose with decreased temperature and fell with slightly higher temperature regardless of the moisture content. Upon thawing of the soil, however, the numbers of bacteria decreased. With a further rise in temperature which made conditions favorable for growth, an increase in bacteria occurred, which reached a maximum by June 19. Two maximum counts were observed during the year—in February and June. Since these counts were of the heterogeneous flora it is not known whether such fluctuations were due to the destruction of one or more types of bacteria or whether some of every type was killed. Lyon, Bizzell, and Conn (8) found the heterogeneous flora to increase if the soil was well frozen but to decrease decidedly after a thaw. Such an increase was explained by Conn (3) as being due to the liberation of a large number of colonies that would not otherwise be recognized. No satisfactory explanation was offered for the sudden decrease after thawing. Vass (10) tried to explain the rise in counts in frozen soils as being due to the breaking up of clumps or colonies of bacteria and not to growth and multiplication.

Vanderleek (9) suggested, from observations he made, that raw material which is available for bacterial decomposition may be responsible for bacterial increase in January, since there was no increase in soils where raw material was absent, but no such explanation was offered when in frozen soils in March an increase in numbers was encountered amounting to from two to four times the original number and which was followed by a decided decrease when the soil thawed. He also reported that a high soil moisture content counteracted the frost action, whereas a low moisture content aided in the depression of bacterial development, and that a sudden severe frost killed most of the bacteria in exposed soil. Harder (6) presents data

which show a close relation between the moisture content and the number of bacteria in soil, yet a distinct retardation by cold which occurred with a high moisture content, was not explained.

One may conclude from such data that there is a decrease in the heterogeneous bacterial flora of the soil sometime during the winter, which cannot be explained entirely by a deficiency of organic matter, by soil temperature conditions, or by moisture as such. It seems that whatever the factor or factors may be that cause a reduction in number of bacteria they do not bring about a sterilization of the soil, although certain definite types of organisms may be almost entirely eliminated from certain soil types, as has been found by Jones and Murdock (7) and by many others.

In an effort to find to what the protective action in soils and solution culture is due, Vasa (10), in a study of the influence of low temperature on *Rhizobium*, states that the concentration of the medium, the length of time of the exposure, and the degree of cold, are the three important factors that determine the power of resistance of the bacteria to low temperature; that the protective action due to the concentration of the medium seems to be effective only in cases in which the eutectic point of the substances in solution is below the temperature of the exposure; and that the death of the bacterial cell when exposed to low temperature seems to be due to the withdrawal of water from the semipermeable membrane or outer layer of the cell. In this connection the work of Giltner and Langworthy (5) may be mentioned. These authors also worked with *Rhizobium* and found that the survival of bacteria in desiccated soil is due in part to a retention of moisture in hygroscopic form. The removal of moisture by desiccation is in a measure comparable to the withdrawal of moisture by freezing.

Very few studies have been made of the seasonal variation of definite species of organisms. An indication of what may happen to a certain genus was suggested by some results which were obtained from a study of the legume bacteria population of the soil. In a report of this study (13), among other things, it was pointed out, through literature cited and through data given, that soils may have a wide variation in the number of *Rhizobium*. The species that produce nodules on *Medicago* ranged in numbers from none in 5 gm. of soil to 100,000 a gram. This variation in numbers seemed to be related to the absence of bases as indicated by the soil reaction. A soil with a reaction further from neutrality than pH 6.0 did not support as large a number of these organisms as one the reaction of which was nearer neutrality.

The findings led to further studies of the effect on nodulation of supplementing the legume bacteria of the soil with artificial cultures (12) and of the value of such cultures as measured by increased crop yields (15). From these studies it was concluded that certain species of *Rhizobium* may largely or entirely disappear from soil, that they do not seem to be greatly influenced by the frequency of the host plant in the rotation, and that to acid soils in particular the addition of more legume bacteria has resulted not only in the formation of a larger number of nodules on young plants but also, in certain cases, in a decided increase in crop yields. It was also observed that the numerically increased nodulation on plants of *Pisum sativum* due to supplementary bacteria became less marked as the plants became older until at blooming time there were decidedly more nodules on the plants which grew in soil where the artificial cultures was not used. This may be taken to indicate a seasonal variation in the number of this genus of bacteria.

The foregoing determinations of numbers of *Rhizobium* and the observations relating to the use of artificial cultures were so striking that it was thought desirable to make a study of the seasonal variation in the number of *Rhizobium trifolii* and of *Rhizobium leguminosarum*, with the view of contributing to our knowledge concerning the subject of supplementary bacteria in relation to the growth of crops.

METHODS

The method of making such a study was essentially the same as that given in a previous publication (13). Samples were taken from .003-acre plats. Quart jars were used to hold a composite sample which consisted of 10 to 12 small samples from the first 3 to 4 inches of soil. To insure uniformity, the composite was well mixed on paper, after which a moisture determination was made. While this was being done the composites were kept in an ice box. In order that the results would be comparable, enough moist sample was taken to give 100 gm. of dry soil. This was then titrated with water in a mortar or dispersed with a mechanical agitator. Dilutions were made from the muddy suspensions. Care was exercised throughout the work to avoid contamination with the legume organism. Portions of the suspensions which were equivalent to a certain quantity of soil, were put on the surface of sterile soil in tumblers where the host plant was to be grown. The tumblers, which held about 200 gm. of sandy soil, were kept tightly covered with paper, which was removed after the plantlets were in need of light. After this period of growth, only 10 or 12 days was required for nodulation to occur sufficiently to determine whether or not the legume organism was present in the quantity of soil used. If contamination occurred after the paper was removed it did not influence the results. Numerous checks were provided and in no case did nodulation occur on the plants in them.

The samples were examined for *Rhizobium leguminosarum* and for *R. trifolii*. To determine the approximate dilutions required for such an examination preliminary tests were made. After suitable dilutions were found they were used in the work herein reported and were modified whenever necessary.

Since it was known that both red clover seed (*Trifolium pratense*) and vetch seed (*Vicia villosa*) carry as contamination (14) the legume organism, the seeds were treated, before being planted, either with a solution of calcium hypochlorite or with concentrated sulfuric acid. Heavy seeding was practiced in order to have adequate plants for inspection. Seeding was usually delayed for 3 to 10 days to permit the establishment of a high legume bacteria content in case the organism was present. After the treated seeds were spread on the surface of the soil in the tumblers they were covered with sterile soil.

The reaction of the soil composite from each plat was determined by the quinhydrone method.

HISTORY OF PLATS

In 1925 and for 9 years previously, plats 751 B, 751 C, 757 B, and 757 C were planted yearly to rye, whereas plats 756 B, 756 C, 756 E, 756 F, 762 B, and 762 C, were planted yearly to oats. In 1926, corn was grown on all of the plats, and in 1927 oats. In 1928, plats 751 B, 756 B, 757 B, and 762 B grew field peas, whereas plats 751 C, 756 C, 757 C, and 762 C grew red clover, and 757 E and F grew cowpeas. Previous to this study the soils of these plats were

known to contain both *Rhizobium trifolii* and *R. leguminosarum*. In the spring of 1928, plats 751 B and 756 B, which grew field peas, had the organism for this crop supplemented with an artificial culture placed on the seed at seedtime. Also plats 751 C and 756 C, which grew red clover in 1928, had their legume

TABLE 1
Seasonal variation in the number of Rhizobium trifolii

Table shows percentage moisture when sample was taken and approximate number of bacteria per gram dry soil.

PLAT	REACTION	TIME OF YEAR WHEN SOILS WERE EXAMINED 1928-29								
			October 11	November 13	December 11	January 22	February 28*	April 11	May 7	June 3
	pH									
751 B	7.50	Per cent Number	22 1,000	22 20,000	26 2,500	23 20,000	39 100,000	21 2,500	25 10,000	17 50,000
751 C	7.40	Per cent Number	12 10,000	23 2,500	26 2,500	23 20,000	36 20,000	21 25,000	25 7,500	18 25,000
756 B	4.95	Per cent Number	11 100	12 500	23 1,000	23 100	21 500	18 50	24 10,000	14 100,000
756 C	4.96	Per cent Number	10 10,000	15 750	22 5,000	20 10,000	20 20,000	21 25,000	24 500,000	15 500,000
756 E	4.83	Per cent Number	10 100	19 100	24 100	23 10	19 10	19 10	24 1 in 1 g.	14 1 in 5 g.
756 F	4.86	Per cent Number	10 100	13 100	23 100	21 10	15 10	19 10	24 1 in 5 g.	14 1 in 2 g.
757 B	7.27	Per cent Number	14 5,000	22 750	26 5,000	25 1,000	27 100,000	21 25,000	25 75,000	17 5,000
757 C	7.31	Per cent Number	15 10,000	22 5,000	26 20,000	24 20,000	23 100,000	21 50,000	25 50,000	17 100,000
762 B	4.88	Per cent Number	11 1,000	15 100	23 100	22 10,000	23 20,000	20 25,000	25 25,000	15 10,000
762 C	4.92	Per cent Number	22 100,000	20 100	23 2,500	23 7,500	12 500	20 50,000	25 75,000	15 10,000

* Ground frozen when samples were taken.

bacteria population supplemented in the same way. Since 1926, all crops have been removed from the plats. In October, 1928, all plats were plowed.

In 1922, plats 751 B, 751 C, 756 B, and 756 C received limestone at the rate of 4,000 pounds an acre, and in 1927 they were again limed at the rate of

2,200 pounds an acre. Other treatments, consisting of potassium chloride and acid phosphate which were applied several years before this study was begun, were given to all plats alike.

RESULTS

The first studies which related to the seasonal variations in the total number of any species of *Rhizobium* in soils were made in 1926. In the following year, additional studies were made. These gave some idea of the number of organisms that may be expected to be present and permitted the development of a technique which was applicable to the work. Beginning in September, 1928, samples were collected regularly about once a month until June 3, 1929. This covered a period when there was no very actively growing plants on the soil. The seasonal variation of *Rhizobium trifolii* is given in table 1.

TABLE 2
Seasonal variation in the number of *Rhizobium leguminosarum*
Figures indicate approximate number to each gram dry soil

PLAT	TIME OF YEAR WHEN SOILS WERE EXAMINED 1928-29							
	October 11	November 13	December 11	January 22	February 28†	April 11	May 7	June 3
751 B*	50,000	20,000	20,000	10,000	20,000	2,500	25,000	75,000
751 C	10,000	20,000	750	2,500	20,000	2,500	7,500	10,000
756 B	50,000	5,000	10,000	20,000	100,000	5,000	75,000	100,000
756 C	10,000	1,000	500	250	250	100	100	250
756 E	250	100	500	100	20	100	1 in 5 g.	1 in 50 g.
756 F	100	250	500	50	500	10	1 in 5 g.	1 in 2 g.
757 B	5,000	5,000	10,000	1,000	10,000	50,000	75,000	10,000
757 C	10,000	500	1,000	20,000	7,500	50,000	25,000	100,000
762 B	5,000	20,000	500	5,000	20,000	25,000	7,500	10,000
762 C	2,500	100	100	2,500	10,000	50	100	5,000

* For percentage of moisture in and reaction of soils see table 1.

† Ground frozen when samples were taken.

The samples which were collected in February were frozen, and the ground remained frozen until about April 1. It should be noted that there is no relation between the soil moisture and the seasonal variation in the number of legume bacteria. What variation is recorded must be assigned to some other factor than moisture even though it varied considerably. Plats 756 E and 756 F are very outstanding in that they have few *Rhizobium trifolii* as compared with plat 757 C or even 756 C, which is within 12 feet of plat 756 E. These two plats at no time during the 8 tests that were made between October and June showed as many as 100 *Rhizobium trifolii* a gram. By January 22, this species had decreased to less than 10 a gram and by June 3 there was probably less than 1 to 5 gm. of soil. This indicates a gradual decrease from October to June. It should be noted that the reaction of these two plats was about pH 4.8.

Other plats did not show such a drastic decline in *Rhizobium trifolii*. There seems to have been a decline in numbers in November and December, a slight rise in January and February, but a decided decrease in April just after a thaw, with an increase from this date to June 3, at which time the numbers were as high as or higher than they were the previous September.

The plats which show quite consistently large counts of *Rhizobium trifolii* are those which have been limed, namely plats 751 B, 751 C, 757 B, 757 C. They have an alkaline reaction.

The samples of soil which were used for the determination of *Rhizobium trifolii* were also used for a determination of *R. leguminosarum*. The seasonal variation is shown in table 2.

It is noted that the soil from plats 756 E and 756 F did not have over 500 *Rhizobium leguminosarum* to a gram at any time between October 11 and June 3, during which time 8 tests were made. There seems to have been a tendency for the organisms to disappear as spring advanced. In June the soil of plat 756 E did not have more than 1 organism in 50 gm. of soil whereas that from 756 F, which adjoins plat 756 E, had not more than 1 organism in 2 gm. The organisms to each gram of soil of plat 756 C were about 10,000 in October, about 250 in January and February, less than 100 in April and May, and about 250 in June. In the remaining plats there seems to have been a decline in the number of organisms in November and December with a tendency for them to increase in numbers in January and February followed by another decline when the soil thawed in April, with a gradual increase from that date to June 3. Excepting plats 756 C, 756 E, and 756 F, the number of *Rhizobium leguminosarum* in June was as large as or larger than it was in October. If the soil of these plats had been examined later in the spring they probably would have shown similar results.

DISCUSSION

The results of this study of the seasonal variation in the number of *Rhizobium* in field soil suggest that the legume bacteria find certain soils more adapted to their existence than others and that soils whose reaction is as low as pH 4.8, at certain seasons of the year, may have as few *Rhizobium trifolii* and *R. leguminosarum* respectively as 1 in 5 or 1 in 50 gm. of soil. These findings are similar to those reported by Bryan (2), who suggested that when a soil becomes as acid as pH 5 the organism for red clover is killed.

The maximum count of either species of *Rhizobium* obtained from each of the 10 plats from which 8 samples were taken between October and June did not occur at the time of any one sampling. Since the growth and longevity of bacteria in soil are dependent upon climatic factors, the physical and chemical make-up of the soil, and the associative action of the edaphon contained therein, such results might have been expected. Bacteria respond to environment which may operate to increase or decrease the population that

may be found at any season. Such seasonal variations as have been recorded in this paper which have to do with *Rhizobium trifolii* and *R. leguminosarum* have also been reported for the heterogeneous bacterial flora of the soil by Lyon, Bizzell, and Conn (8), by Conn (3), by Waksman (11), and by many others.

Since the number of *R. trifolii* and *R. leguminosarum* were determined in the same samples of soil a relative comparison of the numbers of these two species is possible. If all the individual determinations for the organisms for each species are added separately and a comparison is made, it will be noted that there are about 24 organisms of *R. trifolii* to every 11 of *R. leguminosarum*—a ratio of 2.18:1. If such a comparison is made between the organisms in the soil of the four alkaline plats, there are about 89 *R. trifolii* to every 67 of *R. leguminosarum*, or a ratio of 1.33:1; and in the six acid plats about 5 of *R. trifolii* to 1 of *R. leguminosarum*, which is a ratio of 5:1. Apparently *R. trifolii* finds these soils a better habitat than *R. leguminosarum* does. These findings are rather interesting because it is generally believed that the host plants (*Vicia* and *Pisum*) of *R. leguminosarum* will do better on an acid soil than will the host plants (*Trifolium*) of *R. trifolii*. They also suggest an explanation for the observed increase (15) in shelled peas which were taken from these plats in 1928 where the normal bacterial flora in the soil for this plant was supplemented at seedtime with an artificial culture.

The preceding findings are in agreement with data previously published on a study of the legume bacteria population of the soil (13) in which it was pointed out that soils varied in their ability to support *Rhizobium melilotii*, the variation ranging from none in 5 gm. to 100,000 to a gram.

CONCLUSIONS

Soil samples were collected from ten .003 acre plats 8 times between October 11, 1928, and June 3, 1929. The history and reaction of the plats are given. The samples were examined for *Rhizobium trifolii* and *R. leguminosarum*. Definite portions of the soils were used as inoculating material for sterilized soil that was held in glass tumblers. Seeds of *Trifolium pratense* and of *Vicia villosa*, after proper treatment to remove any adhering legume bacteria, were planted. The soils in the tumblers were kept suitable for plantlets by adding the necessary water which was free of the legume bacteria.

The containers holding the inoculated and plated soils were placed in the greenhouse, where favorable conditions were maintained for growth and nodulation. In about 15 days after planting, the plant roots were examined for nodules. The presence or absence of nodules resulting from a series of dilutions established the approximate number of legume bacteria to a gram of soil.

The number of *Rhizobium* organisms of each species studied showed a wide variation. In the soils of plats 756 E and 756 F the number of *R. trifolii* varied from approximately 100 a gram to as few as 1 in 5 gm. The number varied in the other 8 plats from 50 to 100,000 a gram. The number of *R. legumino-*

sarum varied in the soils of plats 756 E and 756 F from 1 in 50 gm. to 500 a gram, whereas in the other 8 plats there were more bacteria of this species. The variation was in one case from 50 to 5,000 a gram of soil and in another case 2,500 to 75,000.

Except in plats 756 E and 756 F there was a decided drop in the number of both species of *Rhizobium* in all plats as the winter season advanced. This drop did not always occur in the soils of the different plats at the same date. As the temperature increased in the spring and conditions became favorable for growth and multiplication, the bacteria of both species increased until they were in most cases as numerous in June as they were in October.

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INTERRELATION OF NUTRIENTS AND SOIL REACTION ON GROWTH AND INOCULATION OF ALFALFA¹

M. C. SEWELL AND P. L. GAINEY²

Kansas Agricultural Experiment Station

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In crop management there are two important questions pertaining to the production of alfalfa on acid soils: first, what degree of acidity limits the growth of alfalfa provided there is an abundant supply of nutrients; and second, what degree of acidity limits inoculation and hence the nitrogen fixed? The latter question is of particular importance where alfalfa is grown for soil improvement as well as for a hay crop.

In order to throw some light upon these questions, several greenhouse experiments have been conducted and are herein summarized.

The first experiment was planned to show whether alfalfa would grow in an acid medium provided it was well supplied with the necessary soil nutrients. To this end, quartz sand cultures were used with nutrient solutions, uniform as to elements and molecular concentration but varying in degree of acidity. The salts contained in the solutions were $\text{Ca}(\text{NO}_3)_2$, KH_2PO_4 , K_2HPO_4 , and MgSO_4 . Traces of ferric phosphate were used to supply iron.

The nutrient solutions were maintained at three different degrees of acidity, namely at pH values of 4.5, 6.0, and 7.0. The following quantities per liter of the various soils solutions were used in order to obtain these reactions:

pH OF SOLUTION	M $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$	M KH_2PO_4	M K_2HPO_4	M $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$

4.5	9.0	4.5	0	4.5
6.0	9.0	3.5	1.0	4.5
7.0	9.0	0	4.5	4.5

The alfalfa was inoculated and planted in 4-gallon jars. A glass tube with glass wool to protect the lower end was inserted in each jar before 24 kgm. of sand was put in. Five jars were maintained at each reaction and plants were finally thinned to five to each jar. A wax seal was applied to check evaporation. A cone in the center of the jar permitted additions of fresh solution. Solutions were changed twice each week, being withdrawn by air suction.

¹ Contribution no. 191 from the department of agronomy and contribution no. 125 from the department of bacteriology.

² Associate professor of soils and professor of bacteriology, respectively.

The withdrawn solutions were tested colorimetrically for any changes in reaction. The reactions were easily maintained within a few tenths of the indicated pH value.

Plate 1, figure 1, represents the growth of alfalfa with the three conditions of reaction. As is shown, alfalfa grew well in a solution having a pH value as low as 4.5 when well supplied with nutrient elements including, of course, calcium.

The second experiment was designed to show the effect of reaction upon symbiotic nitrogen fixation, as measured by growth of alfalfa, where no nitrogen was supplied in the nutrient solutions. Accordingly other quartz sand cultures were prepared as described in the first experiment. To eliminate nitrogen and to vary the acidity it was necessary to use $\text{CaH}_4(\text{PO}_4)_2$ and $\text{Ca}_2\text{H}_2(\text{PO}_4)_2$. Potassium was supplied as K_2SO_4 , and MgSO_4 was used as in the first experiment. The reactions were maintained at pH 4.0, 6.0, and 7.5.

A nutrient solution supplying nitrogen was added until the alfalfa was about 2 weeks old, after which the nutrient solution without nitrogen was substituted.

The effect of reaction when nitrogen is deficient is shown in figure 2, plate 1. After 3 months' growth the plants were washed out of the sand cultures. In the cultures maintained at pH 4.0, the plant roots were stunted and dying, although the top growth was about the same as at pH 6.0 and pH 7.5. The most striking differences evident were in nodulation. At pH 4, there was but one small cluster of nodules in the five plants. At pH 6.0, comparatively few points of inoculation occurred but at these the nodules were present as large grape-like clusters. At pH 7.5, there were a large number of well-distributed small nodules.

These results demonstrate the need of investigation to determine the relationship between reaction with deficient nutrients and symbiotic fixation by the alfalfa plant.

Experiment 3 was a pot culture test with 10 different soils of varying acidities from southeastern Kansas. Alfalfa seed was inoculated and planted in these soils in battery jars. Each soil received the following treatments: (a) no treatment; (b) lime alone; (c) superphosphate alone; (d) lime and phosphate. The plants were allowed to grow about 4 months and then the roots were examined. In untreated soils, known to be deficient in calcium and highly acid, no nodules were present on the roots. Where lime alone had been applied, there were a few nodules. Plants grown in soil receiving superphosphate alone showed better nodulation than where lime alone had been applied. With applications of both lime and superphosphate, nodulation as well as total growth was best.

The experiment shows that a supply of available phosphorus is very important in determining the degree of inoculation obtained with the alfalfa plant grown in a normally acid soil.

In the fourth experiment, alfalfa was grown in an acid soil in 42 large cylinders holding about 165 pounds each, to which varying proportions of lime, superphosphate, and potassium sulfate were added.

Plate 2 shows the effect of the lime and phosphate treatments on growth of alfalfa in this soil. In the light of the results portrayed in plate 1, figure 1, the indications are that the differences in growth between cylinders 1, 2, and 3 are due to some physiological effect of lime upon either the plant or the legume bacteria or both, rather than to its effect upon the reaction of the soil. It is true that the reaction was changed from pH 5.0 to pH 6.5 with the lime treatments. However, figure 1 of plate 1 shows that alfalfa will grow well at pH 4.5, provided the plants receive all the needed nutrient elements. Hence it may be inferred that the increased growth of alfalfa in cylinders 2 and 3, compared with cylinder 1, is due to some other effect of the added calcium.

Cylinder 21 received the same amount of lime as cylinder 3 and in addition superphosphate at the rate of 450 pounds an acre. The reaction of the soil in this cylinder was approximately the same as that in cylinder 3. Here again it is very evident that the addition of a nutritive element, phosphorus, rather than a change in reaction caused the additional growth.

Further proof that nutrients more than soil reaction can affect the growth of alfalfa is presented in plate 3. In this photograph all the cylinders received the same lime treatment, 2,000 pounds an acre, and had practically the same reaction. The variable was superphosphate. Cylinder 3 received no phosphate and cylinders 9, 15, and 21, the phosphate increases in the order given. The beneficial effect of phosphorus here may have been due to the nutritive value of the phosphate or, as indicated in experiment 3, to better nodulation.

The limited data presented in this report indicate, that the reaction of the soil, if within a range of pH 4.5 to 7.0, is a minor factor in affecting growth of alfalfa, all the needed nutrients being supplied; that the reaction may be a very important factor in symbiotic fixation, under conditions of limited nutrients; and that variation in nutrients with constant reactions causes great variation in the growth of alfalfa, which may be attributed in part to the effect of the nutrient on nodulation and fixation of nitrogen.

PLATE 1

FIG. 1. Growth of alfalfa in quartz sand cultures supplied with solutions varying in degree of acidity.

- a.* Solutions having a pH value of 4.5
- b.* Solutions having a pH value of 6.0
- c.* Solutions having a pH value of 7.0

FIG. 2. Effect of degree of acidity on inoculation

- a.* Solution having a pH value of 4.0
- b.* Solution having a pH value of 6.0
- c.* Solution having a pH value of 7.5



FIG. 1



FIG. 2

PLATE 2

EFFECT OF LIME AND PHOSPHATE TREATMENTS ON GROWTH OF ALFALFA IN AN ACID SOIL

Cylinder 1. No treatment

Cylinder 2. Lime, 1,000 pounds an acre.

Cylinder 3. Lime, 2,000 pounds an acre.

Cylinder 21. Lime, 2,000 pounds an acre plus superphosphate 450 pounds an acre

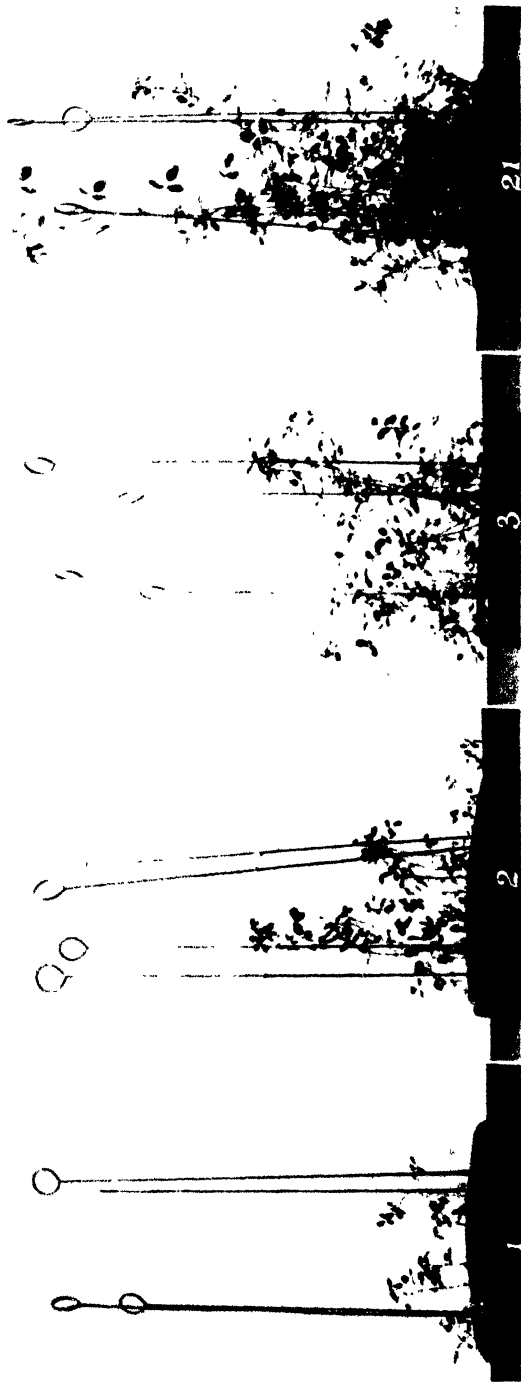


PLATE 3

HOW NUTRIENTS MORE THAN SOIL REACTION AFFECT THE GROWTH OF ALFALFA

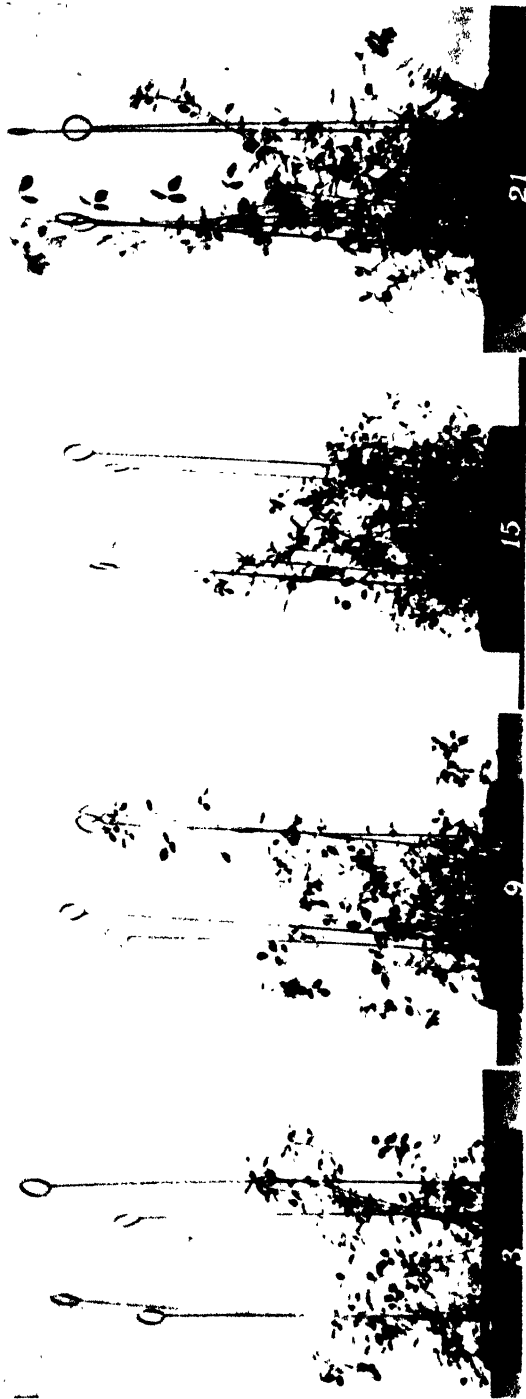
All cylinders have the same liming treatments—2,000 pounds an acre. In addition, the following treatments were given:

Cylinder 3. No phosphate.

Cylinder 9. Superphosphate, 150 pounds an acre.

Cylinder 15. Superphosphate, 300 pounds an acre.

Cylinder 21. Superphosphate, 450 pounds an acre.



DETERMINATION OF CARBONATES IN SOIL

C. J. SCHOLLENBERGER

Ohio Agricultural Experiment Station

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The determination of carbonate native to calcareous soil or residual from liming material applied to acid soil is often required in soil studies. As evidence of the long-continued interest in this determination may be cited the numerous methods described in the literature of soil chemistry. At present there is no procedure which is accepted as standard by American workers. Some of those in use are admitted to be inaccurate, others are too time-consuming or require glass parts that must be specially made or are very complicated. There seems to be a real need for an accurate method that can be carried out in a short time, requires a minimum of apparatus of a kind likely to be found in any well-stocked laboratory or that can be made without difficulty by one with a little skill at glassworking, and is so simple that no elaborate precautions are necessary for high accuracy.

In principle, the estimation of carbonate in soil presents no difficulties; it is only necessary to treat soil with dilute acid to decompose the carbonate minerals and determine the carbon dioxide evolved by any suitable method. However, because of the presence in all soils of organic matter, which is more or less oxidized or decomposed with separation of carbon dioxide in the presence of any considerable concentration of acid, some standard methods for the determination of carbonates are not adapted for use with soils. This action is greatly accelerated by increase in temperature, hence any method which includes boiling the sample with acid at atmospheric pressure for expulsion of carbon dioxide will be grossly inaccurate when applied to soils. This fact is generally recognized, so that aeration with a stream of purified air, often with vigorous agitation as well, to remove evolved carbon dioxide from the soil and dilute acid mixture, is a feature of most methods proposed in recent years.

Aeration is especially adapted to collection of carbon dioxide in a weighed absorption apparatus for a gravimetric determination. Such a procedure is in almost universal use for combustion work but is less satisfactory in the determination of carbonates in soil. To remove carbon dioxide from the dilute acid and soil mixture requires considerable time and a large volume of air aspirated through the solution, which carries considerable moisture into the purifying train. The desiccants used in this require frequent renewal, and there is constant uncertainty as to whether the absorption apparatus may be gaining or losing moisture. At best, it is difficult to weigh the heavy absorp-

tion apparatus with accuracy, so that the theoretical advantages of the gravimetric procedure are seldom realized in practice. Clark and Collins (3) have recently described such a method, and state that from 2 to 7 hours may be required for the absorption apparatus to come to constant weight. In the description of the present tentative method of the Association of Official Agricultural Chemists for carbonates in soil (2), it is stated that 30 minutes aspiration is sufficient, except with soils containing dolomite or limestone resistant to solution. The latter is a volumetric method. In both procedures the carbonate in the sample is decomposed by hydrochloric acid at 1:10 dilution at room temperature and carbon dioxide removed by aspiration with agitation.

With methods employing continuous aspiration there is no means of determining when the last traces of carbonate mineral are decomposed and all carbon dioxide removed. Dolomite or other forms of limestone soluble with difficulty in 1:10 hydrochloric acid at room temperature are of frequent occurrence in the soil in many regions, and are also used as liming materials. It would be very desirable to have some visible indication of completion or decomposition and absorption, as this would result in saving time with samples containing no carbonate or only easily soluble carbonates and would avoid inaccuracy with samples containing resistant limestone. The procedure about to be described has this advantage, as the behavior of the acid and soil boiling in vacuo is characteristic as soon as carbonates are practically completely dissolved, and the great expansion of the bubbles of carbon dioxide arising from even the smallest particle of undecomposed limestone renders them easily visible. The absorption of carbon dioxide by barium hydroxide solution results in a visible film of precipitate on the surface of the solution, a fairly delicate test for completion of absorption. By allowing a short time beyond these points, there can be no doubt that all carbon dioxide which can be obtained has been absorbed. Decomposition and absorption are accomplished in a closed system from which carbon dioxide cannot escape, nor enter from the outside if the details of the procedure are followed. A very accurate titration procedure for the determination of carbon dioxide has been developed. In addition to the foregoing, some hitherto unsuspected sources of error in the determination of soil carbonates are pointed out, and means for overcoming them described.

REVIEW OF PREVIOUS WORK

Probably the most rapid and effective method for the removal of carbon dioxide from sample and dilute acid that can be devised is by boiling in vacuo. As it is possible to accomplish this at a temperature only slightly above that of the laboratory, it is especially adapted to work with soils. A method based on this principle was proposed by Marr (6), and in modified form has long been in use in this laboratory. Gaither (4) substituted a bead tower for the Reiset apparatus employed by Marr, but retained the double titration procedure for the determination of carbon dioxide absorbed by sodium hydroxide solution. Later, carbon dioxide was absorbed in a measured excess of standard barium hydroxide solution in a Meyer absorption apparatus and determined either by filtration and washing, with subsequent

solution in standard acid and titration, or by titrating the excess of barium hydroxide in the presence of the precipitate of carbonate with standard hydrochloric acid and phenolphthalein as indicator (8). The latter procedure has been studied by Truog (10) and shown to be preferable to absorption in sodium hydroxide and determination by double titration. Recently, thymolphthalein has been substituted in the titration of excess barium hydroxide in the presence of precipitated carbonate (9). Long experience with this procedure of titrating excess barium hydroxide has convinced the author that it is amply accurate for all ordinary requirements with only the simplest precautions, and has important advantages over other methods for the determination of carbon dioxide obtained from soil by treatment with dilute acid.

As originally described, Marr's apparatus and the modifications developed later were supposed to require almost continuous operation of the air pump, in order to maintain the vacuum by drawing evolved carbon dioxide through the absorbent solution; none of the designs offered opportunity for absorption in any other way. It was found that there was considerable danger of carbon dioxide being drawn through the absorbent solution and lost, on account of the tendency of this solution to boil because it is under lower pressure than the solution in the evolution flask. This necessitated considerable care and attention. All the forms of apparatus are also of complicated design and require special parts, with numerous joints at which leaks are troublesome. Probably for these reasons, and because it was thought that the use of any heat, however slight, is undesirable in the determination of carbonate in soil, a procedure including the essential feature of decomposition by very dilute acid, boiling and absorption in vacuo, which had been considered as a tentative method by the Association of Official Agricultural Chemists (1), was not retained.

Hutchinson and MacLennan (5) proposed a method based upon that of Marr, requiring only an extremely simple apparatus, consisting of two flasks connected by a wide tube with a bulb to stop foam or spray and stopcock funnels for the introduction of reagents. In their procedure, the sample is placed in the smaller flask and the apparatus exhausted. The absorbent, sodium hydroxide solution, is allowed to enter the absorption flask, and 1:50 hydrochloric acid is allowed to enter the flask containing the sample, in order to decompose the carbonates. No heat is employed, but the reduction in pressure, aided by vigorous agitation, is relied upon to remove all carbon dioxide from the one flask and ensure its absorption in the other. The originators of the method claim that decomposition and absorption can be accomplished in from 20 to 60 minutes.

The author has found that in a similar apparatus and procedure, it is possible to expel all carbon dioxide from the evolution flask and ensure its absorption in barium hydroxide solution in the other flask, within 8 minutes *after all carbonates have been dissolved*, provided the acid and soil mixture is warmed sufficiently to boil at the pressure within the apparatus. This pressure is easily reduced and maintained at a point corresponding to the vapor pressure of water at room temperature, so that the actual temperature in the boiling flask need be but very little higher than that of the room, and may be even lower if a condenser with very cold water is used to connect the flasks. The addition of a condenser to the apparatus described by Hutchinson and MacLennan, therefore, enables one to carry out the procedure of Marr under more favorable operating conditions than were possible with the original apparatus or any modification of it so far proposed. By some other slight changes, the space occupied by the apparatus can be reduced and all the operations facilitated. The most important results of the substitution of absorption of carbon dioxide by exposure to a surface of absorbent solution for absorption by bubbling through the solution are, first, reduction in boiling temperature, and second, insurance of complete absorption. The first result follows from the greater vacuum obtainable in the boiling flask, because the vacuum pump does not work against the hydrostatic pressure of a column of absorbent solution. In the original procedure of Marr, the temperature at which carbonates are decomposed is approximately 50°C. In the Meyer apparatus, the height of liquid is less, and the temperature in the flask with this modification is about 40°C. By using a plain flask as the container for the absorptive solution, the temperature in the boiling flask may be kept without difficulty within the range of 25 to 30°C.

EXPERIMENTAL

Lowering the temperature of expulsion of carbon dioxide is advantageous with respect to lessening attack upon organic matter but is attended by reduction in speed of decomposition of dolomitic limestone. This speed can be increased by using stronger acid, but possibly with the penalty of a corresponding increment of carbon dioxide from organic matter. It should be possible to strike a balance between these factors, and find a procedure which would decompose carbonates quickly with the minimum production of carbon dioxide from sources other than carbonates. With this idea in mind, experiments were undertaken to obtain some definite data on the problem.

The source of the carbon dioxide obtained by the action of acid upon soil which would not be expected to contain any carbonate minerals has been variously explained, but usually vaguely ascribed to decomposition of organic matter. It has been observed that the action of comparatively strong hydrochloric acid is greater than that of the same reagent when highly diluted, or of less dissociated acids such as phosphoric or acetic. No suggestion as to the actual reaction involved has ever been offered, to the author's knowledge. It is his belief that the reaction is largely an oxidation of carbonaceous materials by highly oxidized minerals native to the soil, especially the higher oxides of manganese. Manganese dioxide has been shown to be a common constituent of soils (7). Those soils which contain most, as indicated by ability to decompose 15 per cent hydrogen peroxide, with abundant organic matter also, evolve most carbon dioxide from sources other than carbonates upon treatment with stronger hydrochloric acid or at higher temperature. Addition of chemically pure powdered manganese dioxide to soil and acid results in an increase in carbon dioxide production. In the presence of an effective reducing agent for manganese dioxide, e.g., ferrous chloride, the non-carbonate carbon dioxide obtained from soil upon treatment with dilute hydrochloric acid is greatly diminished.

Hydrochloric acid is the most effective solvent for the carbonate minerals which may occur in soil, but it also reacts with manganese dioxide to form free chlorine, a very active oxidant. This is doubtless the explanation of the fact that hydrochloric acid is apparently more active in decomposing organic matter with production of carbon dioxide than is phosphoric acid, for example. As has just been mentioned, however, addition of ferrous chloride reduces oxidation of organic matter to an almost negligible amount, so that hydrochloric acid plus ferrous chloride is the most suitable reagent for the decomposition of carbonates. From a consideration of data presented in table 1, it is seen that even in the presence of ferrous chloride as antioxidant, increase in either time, temperature, or strength of acid causes slightly higher results, so that it is evidently necessary to operate as near room temperature and with the most dilute acid possible, consistent with complete solution of carbonate in a reasonable period of time.

Experiments were conducted with samples of calcite and dolomite, considered

to be representative of extremes in ease of solution of carbonate minerals likely to be present in soil. Both were ground and sieved to pass 100 mesh and

TABLE 1

Calcium carbonate equivalent of CO₂ obtained from soils by modifications of Marr's method

SAMPLE	(1) 1:50 HCl MEYER APPA- RATUS	(2) 1:50 HCl FLASK	(3) 1:50 HCl + FeCl ₃ FLASK	(4) 1:10 HCl FLASK	(5) 1:10 HCl + FeCl ₃ FLASK	RECOVERY OF CO ₂ FROM 0.2500 DOLO- MITE ADDED TO 20 GM. SOIL GM. CaCO ₃ (THEORY 0.2700)
	m.e./100 gm.	m.e./100 gm	m.e./100 gm	m.e./100 gm.	m.e./100 gm.	
Wooster silt loam, vir- gin: 0-1½ inches, pH 5.1, 173 ml. O ₂ * 1½-5 inches, pH 4.8, 94 ml. O ₂ *	1.61	0.43(23°)	0.18(27°)	0.29(28°)	0.25(28°)	0.2705(30°) 0.2712(28°)
Cultivated, 0-7 inches, pH 4.5, 11 ml. O ₂ *	0.05	(28°)		(28°)		
Same plus 2.5 per cent MnO ₂		(28°)	(28°)	0.15(28°)	(27°)	
Canfield silt loam, virgin, 1½-3 inches, pH 5.6, 98 ml. O ₂ *	1.77	0.15(28°)	0.05(28°)	0.93(28°)	0.13(29°)	0.2705(30°)
Ellsworth silt loam, vir- gin, 0-2 inches, pH 5.4, 50 ml. O ₂ *	1.15	0.28(28°)	0.10(27°)	0.73(28°)	0.18(28°)	0.2705(33°)
Peat, pH 5.2, 38 ml. O ₂ *		0.70(27°) 0.75(31°)	0.60(27°)	1.25(28°) 1.10(34°)	1.00(28°) 6.50(100°)	0.2690(30°)
Peat plus 2.5 per cent MnO ₂		0.70(26°) 0.70(24°)	0.60(26°)	1.85(26°) 3.65(28°) 23.5 (100°)	1.00(28°)	
Hawaiian manganifer- ous soil, pH 5.5†		1.20(36°)	0.05(28°)	1.60(29°)	0.30(28°)	
Toledo silty clay, 0-3½ inches, pH 7.1, 3 ml. O ₂ *		7.43(31°)	7.25(28°)	7.28(31°)	7.13(28°)	0.2697(30°)
Clermont silt loam, 108- 120 inches, pH 8.2, 8 ml. O ₂ *			32.18 per cent		32.20 per cent	

* Reaction determined on 1:1 suspension by use of quinhydrone electrode. Oxygen evolved on treating 1 gm. soil with 20 ml. 15 per cent H₂O₂ for 10 minutes, similar to test proposed by Robinson (7) for presence of MnO₂ in soil.

† Reaction determined as above by use of antimony electrode. Rate of oxygen evolution too great for measurement.

remain on 150 mesh, washed to free from dust, and dried. This particle size was chosen because it is the maximum likely to be present in a soil sample properly prepared for analysis, although it is scarcely practical to grind any

considerable number of samples to uniform fineness greater than this. It was found that 0.25-gm. charges of the calcite were very readily dissolved by 100 ml. of hydrochloric acid at 1:50 dilution (0.23*N*) at 25 to 30°C., solution being complete in about 2 minutes. The rate of solution was slightly retarded by addition of 5 ml. of ferrous chloride solution, about 3 minutes being required. Mixed with 20 gm. of carbonate-free soil, the rate of solution was still less, 5 or 6 minutes being required when solution was aided by boiling in vacuo, within the temperature limits given. Dolomite was very slowly attacked by the reagent at the strength specified, requiring over an hour for solution in the plain acid and nearly 3 hours in the presence of soil and ferrous chloride. By increasing the strength of acid to 1:10 dilution (1.17*N*), it was found possible to insure complete solution of the dolomite in the presence of soil and ferrous chloride in 30 to 35 minutes boiling at 25 to 30°C.

Although data in table 1 appear to indicate that the use of the stronger acid may lead to results slightly too high, it is not certain that the increase in time of action which would have been necessary had the soils contained dolomite, would not have led to plus errors as great even with the more dilute reagent. In the case of the single soil investigated which is known to contain native dolomite, the Toledo silty clay, the results obtained by the use of 1:50 hydrochloric acid, requiring 1½ hours for complete decomposition, are slightly higher than those from the use of 1:10 acid, which decomposed all carbonates in 45 minutes. This soil is high in organic matter, but differs from most of the others studied in that the manganese dioxide content is low, so that the addition of ferrous chloride had little effect upon the results. In this instance, at least, the use of the stronger acid not only resulted in a saving in time, but it apparently decreased attack upon organic matter as well. In other cases where the stronger acid was used with carbonate-free soil, it was observed that practically all the carbon dioxide was obtained during the first few minutes, hence it seems doubtful whether the aforementioned circumstance would be of general occurrence.

In explanation of table 1, it may be said that with three or four exceptions, the soils were selected because they are very acid, but indicate an appreciable carbonate content by the method that has been in use. The results by this method, given in column 1, were obtained by 15 minutes boiling in vacuo with 1:50 hydrochloric acid and absorption of carbon dioxide in barium hydroxide solution, followed by titration in the same way as the others. The temperature of the acid and soil mixture during the boiling was not determined, but is thought to have been about 40°C. Data in columns 2 to 5 were obtained by use of the apparatus and procedure shortly to be described. The temperature at the end of the boiling period was determined in each case. Variations are due to differences in temperature of the room or condenser water, size of flame, and slight leaks of air into the apparatus. In a few instances the apparatus was intentionally only partly exhausted, in order to observe the effect of high boiling temperatures. Except in the case of the Toledo silty clay, all deter-

minations with 1:50 hydrochloric acid were made with a 15-minute boiling period, and all determinations in which 1:10 acid was used, with a 45-minute boiling period. In the last column, recoveries of carbon dioxide from 0.25-gm. charges of dolomite added to 20-gm. samples of soil and decomposed by a 45-minute boiling with 1:10 acid and ferrous chloride in vacuo, are tabulated. In each case, the carbon dioxide obtained from a similar experiment without addition of dolomite (column 5) has been deducted as a blank, and the remainder tabulated as the recovery from the added dolomite. Several careful duplicate determinations of carbon dioxide obtained from dolomite without soil indicated that the calcium carbonate equivalent of each 0.25-gm. charge was 0.2700 gm. The average of the determinations in the presence of soil is 0.27023 ± 0.00023 gm. calcium carbonate.

The $1\frac{1}{2}$ -inch surface layer of the virgin Wooster silt loam contains a large amount of partly decayed organic debris and is also high in active manganese dioxide, as indicated by its ability to decompose 15 per cent hydrogen peroxide. Every modification of procedure obtained some carbon dioxide from this sample. When the acid was added to the soil already wetted with water in the evacuated apparatus, there was noted a flash of foam as shown by soils undoubtedly containing a trace of finely divided carbonate, hence it is possible that this soil really contains a trace of carbonate. It is appreciably less acid than the layer immediately below. Use of strong acid on this sample leads to large error, but this is practically eliminated if ferrous chloride is added with the acid. The figures for recovery of carbon dioxide from added dolomite are both high. The $1\frac{1}{2}$ to 5-inch layer of the same profile is indicated to contain no carbonate when ferrous chloride is added with the acid. The manganese dioxide content of this sample is high. The cultivated sample of Wooster silt loam is deficient in organic matter and low in active manganese dioxide, hence it indicates no apparent carbonate content except by treatment with the stronger acid at a higher temperature. Addition of manganese dioxide to this soil causes slightly high results, but the use of ferrous chloride prevents the oxidizing action. The $1\frac{1}{2}$ to 3-inch horizon of the Canfield silt loam is similar to the corresponding sample of the virgin Wooster silt loam, whereas the surface sample of the virgin Ellsworth silt loam contains less organic matter than the Wooster type, and also less active manganese dioxide, but apparently contains a trace of carbonate.

The three virgin samples indicating a slight carbonate content all show the same increase in carbonate because of substitution of 1:10 hydrochloric acid for the 1:50 acid, ferrous chloride being used in each case. This increase amounts to 0.07 or 0.08 m.c. calcium carbonate to 100 gm. soil and undoubtedly represents the effect of the stronger acid and increased time of action upon the soil organic matter. The difference is quite insignificant, but this is not the case when the corresponding figures from experiments without addition of ferrous chloride are compared. Without the antioxidant, increases in time of action or strength of acid have caused considerable increases in apparent car-

bonate content, and the errors so introduced could not be considered negligible. These differences correspond to differences in the ability of the three samples to decompose hydrogen peroxide, pointing to some factor common to both phenomena.

The peat soil is rather on the border of being a muck and contains considerable mineral matter washed from higher ground. In spite of its acid reaction it appears to contain carbonate. The organic matter is apparently little oxidized by manganese dioxide in the presence of the most dilute hydrochloric acid, but is considerably affected by the stronger acid, especially when heated. Increase in strength of acid has a considerable effect *per se*, apparently quite apart from oxidizing action.

The manganiferous soil¹ is stated to be representative of the chocolate brown, highly manganiferous soils of the Hawaiian Islands. It is high in manganese dioxide and decomposes hydrogen peroxide very vigorously, but appears to be deficient in organic matter. This soil is especially interesting as an example of a case in which two independent lines of evidence, usually confirmatory, lead to a false conclusion. By the ordinary method, this soil is indicated to contain considerable carbonate. It is indicated to be alkaline by the quinhydrone electrode, pH 7.3 at the first reading which can be taken, but with potential drifting very rapidly. If reliance were placed upon the two tests, it would be concluded that this soil is alkaline. Nevertheless, determinations of reaction by the hydrogen and antimony electrodes, and color tests with indicators indicate that the soil is quite acid, between pH 5.5 and 5.7, whereas addition of ferrous chloride to the acid used in the carbonate determination results in only a trace of carbon dioxide being obtained.

The limestone in the Clermont silt loam parent material dissolves readily in 1:50 hydrochloric acid, an indication that it is not of dolomitic character. The results indicate that on this sample, the use of stronger acid is not necessary.

Apparatus

The apparatus with accessories is shown in figure 1. The 200-ml. round-bottomed, short, ring necked Pyrex flask *A* is that in which the sample is treated with acid. The similar 1-liter flask *B* is that in which the CO_2 is absorbed and subsequently titrated. The two flasks are connected by the upright water-jacketed condenser tube *C*, at the upper end of which is a large semi-circular bend attached as a side arm as indicated by the drawing, which is made to scale. The straight part of the condenser tube, continued as the other limb of the T for a short distance above, is closed by a rubber stopper with inserted glass tube, to which is hung by a wire the gauge *G*, also shown in detail. The inner tube of this gauge was made from a piece of graduated pipette, inside diameter about 3 mm. The graduations on the tube enable one to read the

¹ Supplied by W. T. McGeorge, formerly of the Hawaiian Sugar Planter's Experiment Station.

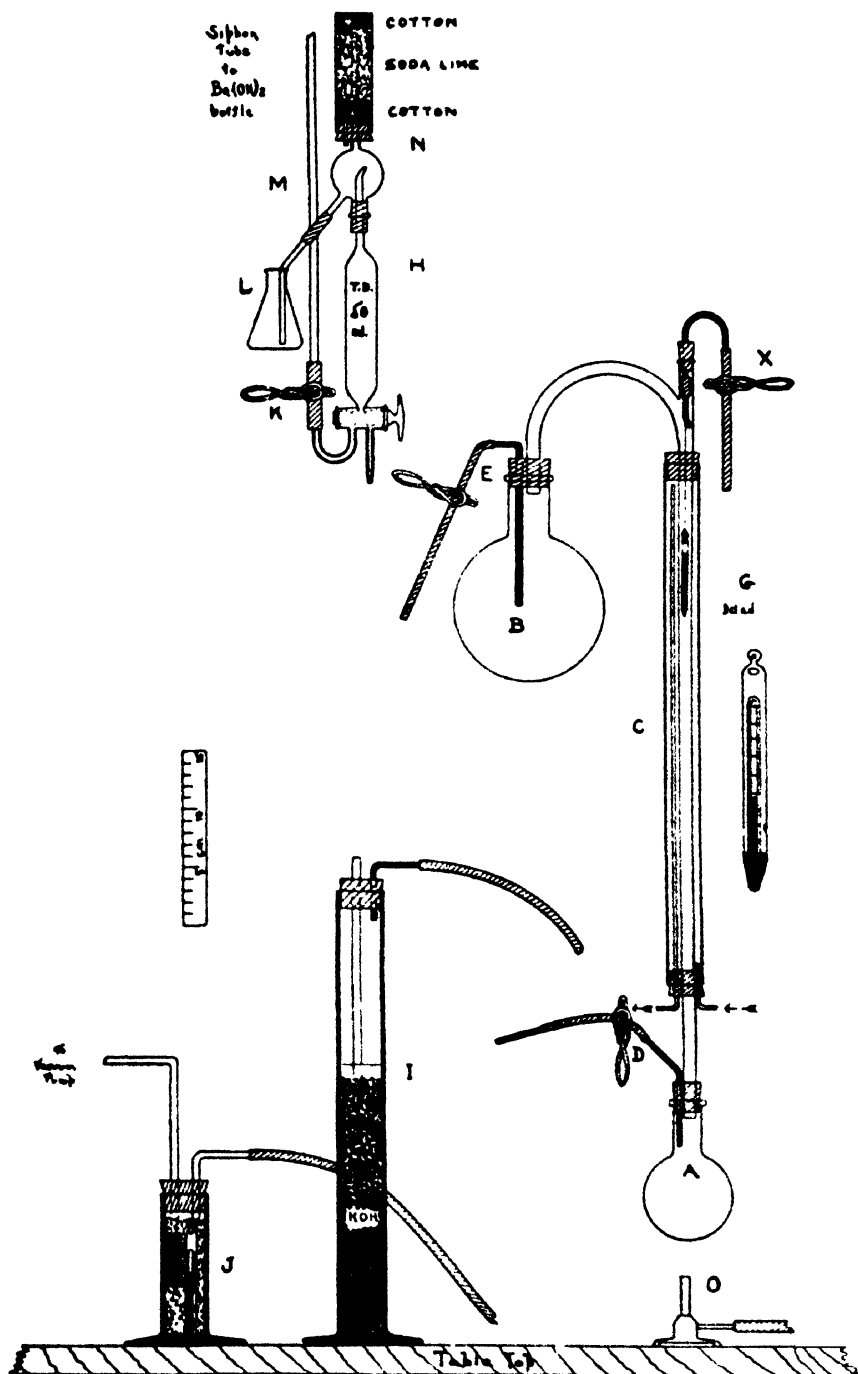


FIG. 1. APPARATUS FOR THE DETERMINATION OF CARBONATES IN SOIL

mercury level with considerable accuracy. Other details of construction will be plain from the drawing. The purpose of the gauge is not so much to indicate the absolute pressure within the apparatus, as to indicate when the apparatus has been exhausted to the practical capacity of the pump, and to give warning of leaks which might otherwise not be suspected. It is not necessary, therefore, to use very great care in filling the gauge with mercury to get every trace of air and moisture from the inner tube. It will suffice to put the mercury in the outer tube, and then put the gauge in a heavy test tube, which is connected to the pump and thoroughly exhausted. By tilting, the mercury is allowed to run away from the tip of the graduated tube, so that there is no hindrance to escape of gas. Under the conditions of practical use, the gauge will never be required to register a vacuum quite as great as this, hence the mercury will always stand at a level where it can be seen and the exact position with reference to the graduations on the tube noted.

The inner tube of the condenser is made from a piece of soft glass tubing, 10 mm. inside diameter and 1 mm. wall thickness. The side arm is of the same tubing. The condenser jacket is a piece of heavy-walled tubing about 25 mm. inside diameter and 45 cm. long. In making the glass parts these dimensions and others which may be taken from the drawing should be followed, as the satisfactory performance of the apparatus may depend upon some of them. The jacket is attached to the condenser tube by means of no. 5 rubber stoppers; both are bored with one large hole slightly off center for the condenser tube, and the lower stopper is bored with two additional holes for glass tubes by which cooling water enters and leaves the condenser.

Especial care should be taken in selecting and boring the two stoppers which connect the flasks to the condenser tube. These stoppers should be of the best quality, red, antimony-cured rubber, selected for soundness and elasticity, and large enough to be forced down into the mouths of the flasks to make a tight joint without risk of being drawn entirely in by air pressure. Each is carefully bored with one large hole 10 mm. in diameter for the condenser tube and one small hole 4 mm. in diameter for the heavy capillary tube of approximately 1 mm. bore for introduction of reagents. These tubes should be long enough to extend down into the flasks as shown. Suitable lengths of 3-mm. heavy-walled tubing are attached to them; this should be stiff enough not to collapse, but not so hard that it cannot be securely closed by the clamps. The most satisfactory clamp is the Day pinchcock pattern.

The apparatus is supported by an upright rod, to which it is secured by one large condenser clamp at about the middle of C. A few turns of adhesive tape about the condenser jacket tube and thick rubber tubing on the jaws of the clamp protect the glass from breakage. The adhesive tape enables the clamp to hold the glass tube without slipping and with less pressure than would otherwise be necessary. If many determinations are to be made, two or three assemblies can be fastened to one rod and all shaken at the same time. Because of the brief time required for a determination with most samples, it will

be found that one man's time is fully occupied by this number. An extra set of flasks for each condenser should be at hand.

Accessory apparatus shown in the figure includes the automatic pipette *H* for delivery of a measured volume of $\text{Ba}(\text{OH})_2$ solution siphoned through *M* from a stock bottle on a shelf above. The overflow from the pipette is caught in the flask *L*. Guard-tube *N*, filled with coarse soda lime, permits only purified air to enter *H* when emptied, hence the pipette rarely requires cleaning. When this is necessary, it can be accomplished without taking down the apparatus entirely. The top part is removed and the body of the pipette cleaned by drawing through dilute HCl , water, concentrated H_2SO_4 with CrO_3 , and is finally very thoroughly washed with water to remove every trace of sulfate before the barium solution is again admitted. Pinchcock *K* is insurance against loss of the entire stock of solution in case the plug should become loose in its shell. *L* and *H* are fastened by clamps to a rod attached to the shelf on which the stock bottle stands, directly above *E*. If only a few determinations are to be made, it will not be worth while to set up an automatic pipette. Excellent results can be obtained by the use of an ordinary transfer pipette, into which the clear $\text{Ba}(\text{OH})_2$ solution is drawn considerably above the mark. The tip is wiped, and the level of the solution adjusted, whereupon the pipette is tilted and the solution caused to retreat from the tip, which may then be inserted into the end of *E* and the solution accurately delivered into *B*.

The bead tower *I* is half filled with glass beads with a strong solution of KOH (30 gm. in 100 ml. water) to cover. Air allowed to enter the apparatus at the end of the procedure, before the contents of *B* are titrated, is drawn through this tower for purification.

Trap *J* is intended especially to protect the vacuum pump from liquid which might be drawn into it, and from dust which may rise from dry soil on sudden application of vacuum. The glass tube connected to the pump ends in a cylinder of wire gauze, closely wrapped with cotton. A gauge is enclosed within the trap.

Any type of vacuum pump which will exhaust the apparatus with sufficient speed is suitable. A "Cenco Hyvac" pump has been in use in this work for two years past, and has given very little trouble.

Reagents

Barium hydroxide solution. Prepare a solution by dissolving the chemically pure crystals at the rate of 65 gm. to a liter of boiled and still hot water. Let stand over night for carbonate to settle. This solution is about 0.4 *N* and is practically saturated at room temperature. Siphon the clear solution into the storage bottle, which is already partly filled with the boiled and cooled water required for dilution to the proper strength. This should be such that one filling of the pipette used to deliver the solution will require slightly less than a burette full of the acid used in the titration. The author makes the solution slightly weaker than 0.2 *N*, delivers it from a 50-ml. pipette and titrates with 0.1 *N* HCl from a 100-ml. chamber burette (M. C. A. No. 3).

Tenth-normal hydrochloric acid. A solution standardized by any of the well-known methods may be expected to have very nearly the theoretical value in carbonate, that is, 1 ml. = 0.005 gm. CaCO_3 . For the highest accuracy, however, it is best to standardize the acid against a sample of pure CaCO_3 carried through the procedure. The water used in the preparation of the standard HCl should have been freed from CO_2 and the solution protected from absorption of any from the air.

Carbon dioxide-free water. Boil distilled water in a large flask or metal can for half an hour, and protect it from the air while cooling and during storage. Dissolved air is objectionable in the water and solutions used in the apparatus, as it reduces the vacuum to some extent. Therefore, CO_2 should be expelled from the water by boiling rather than by aeration. Reabsorption of both CO_2 and air by the water and solutions can be prevented by a layer of pure medicinal paraffin oil about 1 cm. thick, covering the surface. This also prevents the evaporation of solutions in partly filled stock bottles and condensation of water on the exposed walls, and so helps to keep the solution uniform in strength.

Dilute hydrochloric acid. Dilute 1 volume of the C.P. concentrated hydrochloric acid to 10 volumes with CO_2 -free water. This is the 1:10 HCl previously referred to, and is about 1.2 *N*. The 1:50 acid is made by diluting this solution with 4 volumes of water.

Ferrous chloride solution. Put about half a pound of small wire nails in a 500-ml. flask and cover them with hydrochloric acid diluted with an equal volume of water. Add a drop of "antifoam" to prevent foaming over, and set in the hood until action practically ceases. Pour off into a second flask and boil to expel H_2S . Filter and preserve under oil. One ml. should contain about 0.35 gm. FeCl_2 and reduce 0.2 gm. MnO_2 .

"Antifoam." A mixture of equal volumes of light mineral oil and capryl alcohol. A drop or two added from a small oiler to mixtures of soil and dilute acid which cause trouble from tenacious foam rising in the condenser, will cause the foam to break quickly.

Indicator. About 0.5 ml. of a 0.5 per cent solution of either phenolphthalein or thymolphthalein in neutral 95 per cent alcohol may be used, but the two cannot be employed indiscriminately. The latter shows a sharper endpoint in the presence of much carbonate, but has the serious defect of a slow color change, hence the titration must be slowly and carefully performed. It also forms a colloidal precipitate in neutral solutions containing little carbonate, and the "tyndall blue" is likely to be mistaken for the alkaline color of the indicator. If the blue color is very faint and is not diminished by an additional drop of acid under these conditions, in all probability the proper endpoint has been reached. A certain check is to add phenolphthalein, which should show a strong pink color requiring 0.15 ml. 0.1 *N* HCl to discharge if the solution is about 200 ml. volume and was exactly neutral to thymolphthalein. If BaCO_3 from 0.25 gm. CaCO_3 is present, the solution will require the addition of about

0.4 ml. 0.1 *N* HCl to discharge the phenolphthalein color, and this may return on standing, because of loss of CO₂. Therefore, the normality of the acid will depend to a slight extent upon which indicator is used. The difference is to be attributed to solubility and hydrolysis of BaCO₃, which affect thymolphthalein less than phenolphthalein, on account of the higher pH value at which the former is decolorized.

Procedure

Weight out a suitable amount of the sample, which may be 20 gm. or more if low in carbonate, but should not contain much more than the equivalent of 0.25 gm. CaCO₃. Transfer to flask *A* and connect securely. Connect empty flask *B* and attach clamp *E* near the free end of the rubber tube, and also have clamp *X* attached. Connect flask *A* to the vacuum pump through trap *J*, remove clamp *D* and exhaust the apparatus until gauge *G* indicates about 2 cm. Hg pressure within the apparatus, or the greatest vacuum obtainable with water in the apparatus. A satisfactory pump should do this within two minutes. Near the end of the period of exhaustion, connect rubber tube *E* to the tip of the pipette *H*, the body of which is filled with Ba(OH)₂, but with tip and bore of plug empty. Release clamp *E* momentarily, then open the stopcock on *H*. By cautious release of clamp *E*, permit the solution to flow into *B* at about the rate at which such a pipette should be emptied for reproducible delivery, until the last drop of solution has been drawn from the tip of *H*. Close the clamp, then the stopcock, then release the clamp for a moment, in order that the expansion of air in the tip of *H* may drive the solution in the rubber tube *E* into flask *B*. Pinch the rubber tube near its end and remove it from *H*, and without releasing the pressure immerse the end in a 50-ml. beaker filled with CO₂-free water. By releasing clamp *E*, the beaker is emptied rapidly and all Ba(OH)₂ washed from the rubber tube *E* into flask *B*. Needless to say, the flow is stopped while the tube is still filled with water. By this time, the apparatus should be so completely exhausted that the liquid just added is boiling or at least evaporating so rapidly that water is beginning to condense near the top of the condenser *C*, through which a plentiful supply of cold water should be circulating. Attach clamp *D* near the short capillary shown at the end of the rubber tube, stop the pump, and disconnect. Allow about 25 ml. carbon dioxide-free water to enter the apparatus at *X*, adding most of this at once, shaking *A* until the sample is wetted, then adding the remainder of the water to wash dust down from the condenser. Measure out the required amount of 1:10 hydrochloric acid into a small beaker, add 5 ml. ferrous chloride solution and enough water to make 75 ml. Allow this to be drawn into *A* through *D*, cautiously at first lest too much foam be produced. If foam rises into the condenser, a drop of "antifoam" in the open end of *X* with a little water to wash it down will break up the bubbles. If this difficulty is anticipated, the addition may be made to the sample in the flask before the apparatus is connected. However, it is not necessary with many samples, and should not

be used as a routine measure, as it obscures the appearance of minute bubbles indicating incomplete decomposition of carbonate. As soon as foaming moderates, shake *B* to cause the liquid therein to swirl, in order that the crust of carbonate on the surface may be broken up and absorption hastened. Some judgment must be exercised here, as too sudden reduction in pressure within the apparatus as the result of rapid absorption of carbon dioxide may cause the foaming to become unmanageable, especially if the acid and soil have been warmed and no "antifoam" has been added. The apparatus should not be shaken violently—merely moved enough to cause the solution to swirl about. The clamp which grips the condenser jacket near its middle and the thick rubber tubing between readily permit sufficient movement.

When very vigorous evolution of gas has ceased, and most of it has been absorbed as shown by the appearance of the liquid in *B* and the reading of the manometer *G*, light the microburner *O* and adjust the flame to about 5 mm. height. Within a few seconds the mixture in *A* should be boiling vigorously and vapor condensing at about the middle of *C*. As long as considerable decomposition of carbonate continues, the acid and soil will boil quietly. When carbonate is practically all decomposed, violent bumping may be expected. To observe whether decomposition of carbonate is complete, remove the flame, shake the flask, and let it stand quiet for a few seconds. Observe closely whether any small bubbles continue to rise. If none appear within a half minute or so, all carbonate has been decomposed. This test is very delicate, because of the enormous expansion of bubbles in vacuo. Only in the case of samples ground in metal is there any danger of error. An inexperienced operator may possibly mistake peculiar movements on the surface of the liquid, due to the effect of "antifoam" on surface tension, for bubbles quickly breaking. In both cases, however, the error will be on the safe side, so that there is no reason to fear incomplete decomposition. If much carbonate has been decomposed, the boiling should be continued about 8 minutes after this point, in order to be sure that all carbon dioxide has been driven into *B* and absorbed, the absorbent being agitated occasionally meanwhile. At the end of this time, the manometer *G* should read practically the same as it did before the acid was added, indicating that all carbon dioxide has been absorbed, that there has been no leak of air into the apparatus, and that the flow of water through the condenser has been sufficient to prevent accumulation of heat and rise in boiling temperature.

Before opening the apparatus, allow a little carbon dioxide-free water to be drawn in through *D* and *X* to wash the tube and condenser, then connect *D* to bead tower *I* and allow air to enter, slowly at first lest acid be thrown up into the condenser or alkali be drawn over from the tower. While the air is entering, agitate the absorbent in *B*. Disconnect *I*, *A*, and *B* in this order, and close the mouth of *B* with a one-hole rubber stopper plugged with a bit of heavy rod as soon as disconnected, first washing down the outside of the capillary tube extending into the flask with a spurt of water to remove any drops of the absorbent adhering.

Before beginning the titration, shake the liquid in *B*, remove the glass plug, and add the indicator. Insert the delivery tip of the burette filled with 0.1 *N* hydrochloric acid into the hole in the stopper and let the acid run in rather slowly with constant gentle swirling. As the excess of barium hydroxide is neutralized, reduce the rate at which the acid is added until each drop falls separately. As soon as each drop of acid produces a colorless zone in the liquid, discontinue the titration and shake the liquid vigorously, the hole in the stopper being closed with the bit of rod as before. If the amount of carbon dioxide absorbed has been considerable, so that the amount of acid required up to this point is much less than the blank, sufficient carbon dioxide-free water should be added to increase the volume of the solution to that of a blank determination. Neglect of this precaution may cause low results, because the high pH value at which the indicator turns requires an appreciable concentration of barium hydroxide. Continue the dropwise addition of acid from the burette until the indicator color is largely, but not entirely, discharged, then shake the flask again. Following this, some color should still remain, requiring 2 or 3 drops of acid to discharge completely. Deduct the burette reading from that of a blank determination conducted in the same way except that no sample is included, in order to get the volume of standard acid corresponding to the carbonate content of the sample.

Notes on the determination

Samples of peat soil and leaf mold are most troublesome, as the light and powdery material is prone to rise in dust when water is first added, and also foams persistently when acid is added. By the procedure described, these troubles can be overcome. Many of the precautions are not necessary with ordinary mineral soils.

During the latter part of the boiling period, after most of the carbon dioxide has been expelled, the mixture is likely to bump with great violence and be thrown high into the condenser. It is for this reason that the apparatus is made tall. Such a very violent bump always follows a period of perfect quiet, during which the solution is becoming slightly superheated. Gentle shaking will prevent violent bumping. At each bump, a loud click or "water hammer" is heard, leading the inexperienced operator to fear that the apparatus will collapse at a repetition. During the course of thousands of determinations, the author has had only two or three flasks cracked from this cause. In each instance a star-shaped crack appeared in the side of the flask, was noticed at once, and the determination saved by smearing grease upon the crack to stop the entrance of air. The only instance in which any part of the apparatus collapsed from air pressure was when a flat-bottomed flask was tried. Only spherical flasks can be depended upon to stand the pressure.

The condenser tube should not be constricted at the lower end, lest liquid thrown up and condensate be unable to return past the ascending vapor, and finally be thrown into *B*.

The pressure within the apparatus should be watched closely, as a rise indicates that the boiling temperature is rising, with risk of obtaining carbon dioxide from the decomposition of organic matter. Increase in pressure may be due to a leak, too large a flame, too warm condenser water, or ineffective absorption of carbon dioxide. If not due to leakage, the manometer reading should decrease when the flame is removed and the apparatus shaken. Poor quality, ill-fitting, or badly bored rubber stoppers are the chief cause of leaks; tubing has given little trouble.

If too large a flame is used, or the condenser water is warm, vapor may pass into *B* and condense there. Except that it is an indication that the apparatus is not properly managed, and that the temperature is rising, this does no harm. The vapor carries no hydrochloric acid, with the possible exception of a trace in spray.

The question of the optimum strength of acid to be used for the decomposition of the carbonates in a particular sample, or to be used for routine work, is a matter of judgment. One to fifty acid is entirely too weak for samples containing dolomitic limestone, and is probably weaker than necessary for routine use. Hydrochloric acid at 1:25 dilution with ferrous chloride will probably be more generally satisfactory. For samples containing dolomite, 1:10 acid is advised, always with ferrous chloride. If a determination has been started with weak acid and the continued evolution of carbon dioxide after a reasonable time indicates that dolomite is present, the strength of acid in the boiling flask is easily increased, best by the addition of 1:2 hydrochloric acid.

The time required for a determination will depend upon the nature of the carbonate mineral in the sample, the fineness to which it is ground, the strength of acid used for decomposition of carbonates, and the amount of agitation by boiling or shaking. If the carbonate is calcite, it will be decomposed in a short time, even with the 1:50 acid. If it is a very resistant limestone or dolomite, a 1:10 acid will be desirable. If a sample of this kind is ground to pass 100 mesh, decomposition should not require more than 45 minutes, and probably much less in most cases. The time required for complete expulsion of carbon dioxide from the acid and absorption in the barium hydroxide is about 8 minutes, when aided by several gentle shakings. Breaking the vacuum and dismantling the apparatus require about a minute. After some experience, the titration should not require more than 5 minutes. One can demonstrate in 7 or 8 minutes that a sample contains no carbonate, or only a trace, and complete a determination under the most favorable conditions in about 15 minutes. No properly prepared sample should require more than an hour for an accurate determination of the carbonate content. One man can handle three or four assemblies of apparatus, and with ordinary samples his time will be fully occupied.

Although the procedure described was developed for work with soils, there seems to be no reason why it should not be adapted to the determination of carbonates in other materials. For use with samples from which volatile acids other than carbonic are evolved under the conditions of the experiment, it

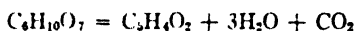
should be possible to neutralize the absorbent, filter and wash the precipitate, dissolve in excess standard acid, and titrate for an accurate determination of carbonate. Experiments have shown that by this procedure accurate results are obtained when hydrogen sulfide accompanies carbon dioxide. If the sample contains sulfite as well as carbonate, it should be possible to absorb both gases, add an oxidizing reagent and excess acid, and reabsorb carbon dioxide alone.²

SUMMARY

A simple apparatus and procedure for determination of carbonates in soil are described.

² Addendum, July 15, 1930.—Since this paper was accepted for publication, Dr. E. C. Shorey, of the soil fertility laboratory, Bureau of Soils, U. S. Department of Agriculture, has informed the author in a personal communication, dated July 10, that he believes the presence of uronic acids in soil to be a source of error in carbonate determinations. He has obtained evidence that substances of this class are of common occurrence in soils, and that they are more readily decarboxylated in the presence of quite dilute acids than has been realized.

The decarboxylation reaction



is quantitative, at least with respect to carbon dioxide, when uronic acids are boiled for a minimum of five hours with 12 per cent HCl. The furfuraldehyde is obtained in amount less than demanded by theory, but collection and determination of CO₂ evolved have been made the basis of a method for determination of uronic acids.* In a later paper,† it is stated that in the presence of very dilute acid the uronic acid is decomposed, but reversion products are formed which are very resistant to further decomposition, and the yield of CO₂ is no longer quantitative. In this case the course of the reaction is not a function linear with time, but proceeds at a rapidly diminishing rate. This seems particularly unfortunate, since it detracts from the value of an obvious means for correcting the error in carbonate determinations. With a constant rate of decomposition, it would be possible to make a second determination with twice the time allowed for the action of the acid, and any increase in CO₂ obtained during the doubled time would correspond to the blank to be applied to the first determination.

The authors of the latter paper state that with a decrease in temperature below 100°C. the rate of decarboxylation in the presence of dilute acid falls off rapidly, but present no data in support of the statement. As has already been mentioned in discussion of the data presented in table 1, increase in strength of acid and in time of action has been found to increase the amount of CO₂ obtained by the procedure described, even when ferrous chloride had been added to prevent an oxidizing action. In the case of three similar virgin surface soils containing considerable leaf mold and partly decomposed forest debris, a five-fold increase in strength of acid and a three-fold increase in time resulted in a constant increase in CO₂ obtained, about 0.08 m.e. per 100 gm. soil. This is probably the extent of the error due to decarboxylation from increase in severity of treatment. Even granting that these soils are entirely free from carbonates, and that the whole of the apparent carbonate content is due to decomposition of organic matter, the marked effect of ferrous chloride indicates that under the conditions of these experiments decarboxylation is a minor source of error compared to oxidation.

* DICKSON, A. D., OTTERSON, HENRY, AND LINK, K. P. 1930 A method for the determination of uronic acids. *Jour. Amer. Chem. Soc.* 52: 775-779.

† LINK, K. P. AND NIEMANN, CARL 1930 The action of weak mineral acids on uronic acids. *Jour. Amer. Chem. Soc.* 52: 2474-2480.

Carbon dioxide evolved from carbonates on boiling the sample with dilute hydrochloric acid containing ferrous chloride, below 30°C. in an evacuated apparatus, is absorbed in excess standard barium hydroxide solution under conditions ensuring complete absorption, and the excess barium hydroxide is titrated in the presence of the precipitated carbonate.

A study of the reasons for high results in the determination of carbonates in soil is reported. It is shown that oxidation of organic matter to carbon dioxide by reaction with manganese dioxide native to the soil and added acid may be a factor that is in many cases important even with the most dilute acid at room temperature. The addition of ferrous chloride to the acid used for decomposing soil carbonates is proposed as a remedy for this source of error.

As ferrous chloride does not entirely prevent evolution of carbon dioxide from soils containing no carbonates when treated with comparatively strong acid at higher temperature or for a longer time, the importance of conducting the determination at the lowest temperature and with the most dilute acid that can be used, consistent with complete decomposition of carbonates in a reasonable time, is stressed.

Special advantages claimed for the apparatus and procedure are greater simplicity, rapidity, and accuracy. Carbon dioxide from sources other than carbonate minerals is minimized, completion of decomposition can be recognized, and complete absorption ensured.

Application to determination of carbonates in materials other than soil is suggested and procedures outlined.

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RELATION OF NITRATES IN SOILS TO THE RESPONSE OF CROPS TO POTASH FERTILIZATION:¹ I. FACTORS CONTRIBUTING TO THE UNPRODUCTIVENESS OF "ALKALI" SOILS IN ILLINOIS

O. H. SEARS

University of Illinois Agricultural Experiment Station

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The capacity for crop production of thousands of acres of more or less unproductive soil, commonly known as "alkali," which are located in the northern half of Illinois, may be greatly increased by the application of potash salts. The need for potassium on these soils has existed for many years and was recognized previous to 1912.

On other soils, particularly in the southern third of the state, the need for potash salts appears to be developing and is usually associated with the repeated growing of sweet clover on the land.

The results reported in this paper were obtained in studies of the first group of soils, begun in 1923 to determine the reasons for crop response on these soils where potash salts were applied. As a result of many laboratory tests, greenhouse studies, and field trials, it is concluded that two factors contribute to the unproductiveness of these soils: first, a deficiency of available potassium; and second, an excessive accumulation of nitrate nitrogen.

The first of these conditions is associated with the presence of a high lime content in a large percentage of cases, although in some instances a low amount of total potassium exists.

The second factor is the result of conditions favorable for nitrate formation, particularly the presence of calcium carbonate and an abundance of readily nitrifiable organic matter.

The following observations are presented in support of the conclusion that these two factors play an important part in the responsiveness of these soils to applications of potash salts.

The "alkali" soils of Illinois, occurring in tracts which vary in size from a few square rods to many acres, are usually found in connection with swamp land that has been reclaimed by drainage and brought under cultivation. Consequently, they are usually high in organic matter and they contain an abundance of carbonates. The first one or two crops grown are ordinarily satisfactory, but subsequent yields are disappointing. Corn appears to be affected most seriously. It germinates well and makes a normal growth for a very few weeks, after which it ceases to grow, turns yellow, and finally has the appearance of being badly diseased.

¹ Published with the approval of the director. Contribution from the department of agronomy. This paper was submitted to the faculty of the University of Illinois in partial fulfillment of the requirements for the degree of doctor of philosophy.

Small grains produce an abundant vegetation but lodge badly, and as a result a low yield of shriveled grain is produced. Sweet clover, however, makes a splendid growth.

After adequate drainage has been obtained, the capacity for crop production on these soils is greatly increased by the application of potash salts and by the use of straw, corn cobs, or manure, particularly horse manure. In fact, after such treatments, these soils frequently produce larger and better crops than the fertile land surrounding them.

A whitish deposit on the surface of these soils, when dry, is a characteristic feature which has led to the general adoption of the term "alkali" for the designation of these unproductive areas. Although it is generally recognized that the alkali differs from the black alkali of the arid regions, opinions differ as to the cause of the unproductiveness of these soils.

Unproductive soils, similar in character to those in Illinois, are known to occur in Iowa, Wisconsin, Indiana, and to some extent in other states. Although investigators in these states generally recognize the same characteristics and have improved the crop-producing capacity of these soils by the same kinds of treatment, there is considerable difference of opinion as to the cause of their unproductiveness.

King and Jeffrey (13) believed that some injurious principles exist in the soil water and that the beneficial influence of potassium-containing salts could not be in the direction of supplying needed potash for plant-food. They state that they were much surprised to find that no perceptible influence was exerted by liquid manure, whereas a very large increase was realized on the plots receiving solid manure.

Conner and Abbott (7) were of the opinion that a lack of available potassium is the factor limiting the crop yields on the Indiana soils of this character, and that the value of straw, corn cobs, and similar materials is due entirely to the potassium contained therein. They stated that "wheat and oats straw contain from seven-tenths to over one per cent of potash—practically all soluble in cold water." They concluded from this that it is obvious that no other explanation is necessary of why wheat straw is a beneficial treatment for peat soils deficient in potash. It may be added in this connection that many alkali soils not deficient in total potassium give a marked increase in yield when straw applications are made.

Bancroft (2) concluded that the presence of calcium bicarbonate is the chief soluble salt in the alkali soils of Iowa, although he agreed that the presence of other salts in addition to this compound may prove injurious. It is stated that drainage and the application of manure will remedy alkali conditions in Iowa, whereas potassium and calcareous clay have practically no influence on the reclamation of such soils. As no crop yields are reported, it is assumed that these conclusions are based upon chemical analyses alone. Analyses of the leachings of soils receiving various treatments are given, but no mention is made of the nitrate content of the soil.

Hopkins, Readhimer, and Fisher (11) were of the opinion that these soils were not improperly called "alkali," and that magnesium carbonate was the toxic substance responsible for the low crop yields. They concluded that plenty of tile, laid deep and made to work, is all that some of these alkali spots need to make them grow good corn. Subsequent investigations (18) at the Illinois Experiment Station on other kinds of soil indicate that magnesium toxicity is negligible even though a relatively high proportion of magnesium carbonate is present.

In planning the experiments which are presented here, consideration was given to the possible causes of the unfavorable soil condition or conditions resulting in low crop yields. It is obvious that the unproductiveness could be due to the absence of an essential factor, to the presence of an unfavorable influence, or to a combination of both. The treatments used in an effort to improve these soils were selected with these things in mind.

Recognizing the limitations of laboratory work, and appreciating the fact that greenhouse studies should be supplemented by field trials, the author

employed all three methods in the investigations herein reported. Furthermore, in order that any conclusions which were reached might not be too limited in their application, 10 soils from 9 counties were investigated during the progress of the work.

GREENHOUSE EXPERIMENTS

Effects of crop residues and mineral fertilizers

Preliminary results obtained by F. H. Maxfield, as a part of a student's thesis, indicated that potash salts were effective in increasing crop yields on alkali soils. Either wheat or oats straw used as a mulch was more effective, however, than the chloride or sulfate of potash, even though the straw and

TABLE 1

Weights of lettuce and wheat grown under greenhouse conditions on black sandy loam from Cass County

SOIL TREATMENT		WEIGHT OF CROPS		
Kind	Amount*	Lettuce	Wheat	
			Grain	Straw
		gm.	gm.	gm.
None.....		1.05	4.25	8.75
K ₂ SO ₄	200	1.40	7.50	14.35
KCl.....	200	1.85	6.09	13.26
K ₂ CO ₃	200	1.08	7.62	12.43
Wheat straw.....	5 tons mulch	2.40	8.20	13.06
Sweet clover.....	1 ton mulch	1.08	10.58†	17.70

* Unless otherwise stated, all soil treatment is given in terms of pounds an acre (2,000,000 pounds).

† Additional treatment of 200 pounds potassium sulfate and 500 pounds superphosphate.

potash salts contained the same amount of potassium. Sweet clover, on the other hand, was ineffective (table 1).

It appears that the use of potassium-containing salts had a beneficial effect upon the growth of lettuce and wheat. When judged by the yields of lettuce, the chloride appears to be the best of the three salts tested, and potassium carbonate the least effective. The residual effect upon the subsequent wheat crop, however, was greatest where the sulfate had been used. This difference may be accounted for by the fact that the chloride had produced the biggest crop previously. When results obtained in subsequent trials are considered, there appears to be little choice between the chloride and sulfate for use on this soil. In nearly all cases the potassium carbonate was somewhat less beneficial than either of the other two potash salts. This is probably due to its tendency to increase the alkalinity of an already too alkaline soil.

It is of interest to find that wheat straw had a more marked influence upon

the yield of lettuce than any other treatment. There are at least three possible explanations for the increased yields where the straw is applied: first, the straw served as a source of readily available potassium; second, the organic matter added in the straw reduced the toxicity of the soil by absorption of unfavorable constituents; and third, favorable changes in the microorganic population of the soil were brought about through the addition of food materials in the straw.

The investigations of many workers have proved that straw serves as a source of readily soluble potassium but it seems unlikely that the greater benefit resulting from this treatment is due solely to the potassium which it contains, since both the potassium chloride and carbonate treatments contain a larger amount of this element than the straw.

Little significance can be attached to the second point as an explanation of these observed differences in crop response because sweet clover not only furnishes potassium but also supplies organic matter; nevertheless, it had no beneficial effect upon crop yields.

The yields of both lettuce and wheat suggest that in addition to the benefit derived from the potassium contained in the straw, a biological factor is to be considered. Several investigators (6) have proved that applications of straw and other cellulosic materials lower the nitrate nitrogen content of soils. This decrease is not a permanent effect, however, for the soil returns to its normal condition after a few weeks. Since these alkali soils readily produce nitrate nitrogen, it is conceivable that in the absence of certain essential elements, such as potassium, an accumulation of nitrate nitrogen is toxic, either in itself or by lowering the resistance of the plant so that other unfavorable influences become increasingly detrimental. Under such a condition, the beneficial effect of wheat straw may be attributed to two effects; namely, the addition of available potassium and the diminution of soil nitrates. Sweet clover, on the other hand, could not be expected to exert a beneficial effect because any benefit due to soluble potassium which it contained would tend to be offset by the accumulation of nitrates induced by the sweet clover.

In the residual effect upon the wheat yields, the same tendencies are evident. The influence of potassium, whether applied as salts or in the straw, is still apparent, but the difference between the straw and potash treatments is considerably decreased. This would be expected if the effect of the straw is due partially to biological activities, for, as previously stated, the nitrate reduction is only temporary. The method of applying the straw, however, is an important factor determining the residual effect upon nitrate accumulation. When it is incorporated with the soil, as compared with the mulch method, a more pronounced immediate reduction of the nitrate nitrogen content is brought about, followed shortly by a decided nitrate accumulation. When it is used as a mulch, a less pronounced immediate effect is obtained which persists longer before excessive nitrate accumulation again occurs.

In general, the soil from Whiteside County gave results similar to those ob-

tained with the soil from Cass County (table 2). Of the three crops grown, corn is decidedly the most responsive to potash fertilization, a fact which is well recognized from general observation. The lettuce crop, however, is the only one responding to phosphate treatment, giving increases which are quite striking, particularly when used in addition to a potassium-containing salt. This observation well illustrates the possibility of error when an attempt is made to apply the results obtained from one observation to other conditions. As subsequent data will show, no evidence has been obtained indicating that corn grown on this kind of soil is responsive to phosphate treatment.

The value of straw is again strikingly demonstrated, especially where it is used in addition to the potassium salts. The treatment consisting of sweet

TABLE 2

Weights of lettuce, corn and wheat grown under greenhouse conditions on black sandy loam from Whiteside County

SOIL TREATMENT		WEIGHT OF CROPS			
Kind	Amount*	Lettuce	Corn	Wheat	
				Grain	Straw
		gm.	gm.	gm.	gm.
None		0 93	45 5	7 2	17.5
K ₂ SO ₄	300	1 35	106 0	8.3	20.3
KCl	300	0 93	112.5	8.2	19.6
K ₂ CO ₃	300	0 92	91.5	9.2	17.1
NaCl	300	0 85	49.0	7.5	19.7
Superphosphate	500	1.35	42 0
K ₂ SO ₄ superphosphate	300	2 25	116 5	7.9	20.9
	500				
Wheat straw.....	2 tons	1.28	207 5†	9.1	23.8
Sweet clover.....	1 ton	1.04	131 0†	9.4	23.4

* Unless otherwise stated, all soil treatment is given in terms of pounds an acre (2,000,000 pounds).

† 250 pounds of K₂SO₄ and 500 pounds superphosphate in addition to previous treatment.

clover, superphosphate, and potassium sulfate cannot be considered any more effective than the corresponding treatment without the sweet clover, whereas, the straw in the same combination has had a very marked influence on the corn yields, again indicating that it owes its value in part to factors other than the potassium content. The residual effect upon the succeeding wheat crop is very small. Two conditions contribute to such a result. First, wheat appears to be less seriously influenced by the toxicity of these soils than is corn, consequently it is less responsive to the usual effective treatments. Second, the residual effect of straw treatment could not be expected to be large if its value depends upon its effect on the accumulation of nitrates in the soil, in addition, of course, to the potassium which it supplies.

Besides the effect upon crop yields, both straw and potash treatments had a marked influence upon the appearance of the plants. In the absence of soil treatment, a browning and curling of the leaf tip occurred. In some cases this condition progressed to the complete killing of many leaves. These symptoms are unlike those obtained where potassium-deficient nutrients are used for the growing of corn. The leaves of the corn in the latter case are striped but not brown and crinkled. Apparently some factor in addition to a potassium deficiency affects the leaf reaction in these soils.

Many experiments have shown that sodium chloride is effective in increasing crop yields on many potassium-deficient soils. This response has been attributed to a base-exchange phenomenon and to the direct substitution of

TABLE 3
Weights of corn grown under greenhouse conditions on deep peat soil from Cass County

SOIL TREATMENT	WEIGHT OF CORN CROP			INCREASE OVER CHECK
	1st jar*	2nd jar*	Average	
	gm.	gm.	gm.	per cent
None.....	44.8	55.0	49.9	...
2.5-ton straw mulch.....	116.5	115.3	115.9	132.2
5.0-ton straw mulch.....	151.1	153.8	152.5	205.6
Ashes from 2.5 tons straw.....	89.8	75.3	82.6	65.5
Ashes from 5.0 tons straw.....	91.0	111.3	101.2	102.8
1-ton sweet clover mulch.....	94.3	97.3	95.8	92.0
3-ton sweet clover mulch.....	130.3	132.3	131.3	163.1
Ashes from 1 ton sweet clover.....	90.3	86.2	88.3	77.0
Ashes from 3 tons sweet clover.....	113.0	132.3	122.7	145.9
200 pounds potassium chloride.....	112.7	113.0	112.9	126.3
5.0-ton straw mulch plus 200 pounds potassium chloride.....	163.0	161.0	162.0	224.6
Ashes from 5.0 tons straw plus 200 pounds potassium chloride.....	143.3	140.7	142.0	184.6

* Average of three successive crops upon the same jar.

potassium by sodium in the nutrition of the plant. In this experiment, however, sodium chloride failed to affect any of the crop yields materially.

Comparison of straw and sweet clover with their ashes

The two previous experiments indicated that the value of straw for the improvement of these alkali soils was not confined to the potassium it contained. If, in addition to supplying this element, a beneficial influence was exerted through a lowered nitrate nitrogen content as a result of microorganic activity, it follows that unburned straw should have a more marked effect upon crop yields than would its ashes. On the other hand, sweet clover would not be expected to be more effective than its ashes since it would supply only one factor—potassium—and would not lower the amount of soil nitrates, but rather tend toward increasing them.

Table 3 gives the results of an experiment designed to test this hypothesis.

These results were obtained on a soil located one-fourth mile from the Cass County soil used in the previous experiment. It is somewhat higher in organic matter, but, as can be seen from a comparison of the results, responds to treatment in a manner similar to that of the other soil. .

It may be seen that a mulch consisting of 2.5 tons of straw to the acre was less effective in increasing corn yields than was a 5-ton application, and that a straw mulch was twice as effective as the ashes from the corresponding amount of straw.

In the case of sweet clover, however, there was little difference between the mulch and the ashes. On a percentage increase basis there appeared to be an advantage in favor of the unburned sweet clover, but an inspection of the duplicates shows that the difference was within the range of experimental error. It appears, therefore, that the wheat straw supplies a factor favorably influencing the growth of corn and which may be lost by burning, whereas the sweet clover is lacking in this factor. Furthermore, 2.5 tons of straw supplied in the form of a mulch results in a larger increased corn yield than an amount of potassium chloride supplying twice as much potassium. The results are in agreement with those reported in table 2 for another soil.

Effect of growing sweet clover upon subsequent yields

Sweet clover makes an excellent growth on alkali soils in Illinois. It would be reasonable to assume that if a potassium deficiency were the sole factor limiting yields on these soils, the growing and plowing under of a sweet clover crop should increase crop yields, the beneficial effect being obtained by the release through decomposition of the potassium contained in the sweet clover plants. Field observations on a Cass County farm failed to justify such an assumption, however, for the poorest corn in the field was found on the area where the largest amount of sweet clover was plowed down. This condition might have been attributed to a moisture effect, were it not for the fact that splendid corn was grown under the same conditions where potassium chloride was applied. It seemed desirable, therefore, to determine under controlled greenhouse conditions the extent to which sweet clover would restore the productive power of these soils. Consequently, an experiment was begun in which biennial white blossom sweet clover was grown in some jars while other jars containing the same kind of soil were allowed to remain fallow although kept at the same moisture content. When the sweet clover had attained a height of about 18 inches, tops and roots were returned in some cases to the soil in which they grew, whereas in other cases they were removed and placed in fallow soil. All of the jars were planted to corn, which produced the yields reported in table 4.

It cannot be said that the growing of sweet clover on this soil had a marked influence on its productive capacity, since the average yield of jars 11 and 12 with sweet clover is little above the average of the checks, jar 12 being but 0.9

gm. heavier than jar 13. For a similar comparison, jars 3, 4, 9, 10 may be considered. When the variation between duplicates is considered, there is little evidence to indicate that the sweet clover, in addition to the potassium chloride, has had any pronounced effect. It is evident, however, that the sweet clover crop has taken something of value, apparently potassium, to the corn crop from jars 5 and 6 and added it to jars 7 and 8. In this connection, it is of interest to note that the average decrease in yield on the soil from which the sweet clover has been removed is 52 gm. and that the gain where the sweet clover tops were added is 40 gm. The results from this single experi-

TABLE 4

Weights of corn grown under greenhouse conditions on black sandy loam from Cass County, as influenced by a sweet clover green manure crop

JAR NO.	SOIL TREATMENT		CORN WEIGHTS FOLLOWING SECOND TREATMENT	
	First	Second	Average	Individual
			gm.	gm.
1	Fallow	None	133.0	130.7
2				130.8
13				137.4
3	Fallow	KCl—150 pounds an acre	189.1	178.7
4				199.5
5	Sweet clover	Sweet clover removed—tops and roots	81.0	80.0
6				82.0
7	Fallow	Sweet clover tops from 5 and 6 added	173.0	173.0
8				172.9
9	Sweet clover	Sweet clover returned—KCl—150 pounds an acre	188.8	191.5
10				186.1
11	Sweet clover	Sweet clover returned	141.6	144.7
12				138.5

ment are too meager to warrant a statement to the effect that sweet clover will not improve this kind of soil. The data are suggestive, however, and indicate that on this one soil any benefit which is derived from an increased availability of potassium brought about by the growing and returning of the sweet clover may be nullified by the increased nitrate nitrogen which accumulates as a result of the rapid decay of the sweet clover.

Relation of temperature and moisture to crop yields and nitrate accumulation

It is a common observation that the yield of crops produced on these alkali soils varies more widely from season to season than on most soils. The most

productive areas appear to lie either in the most poorly drained part of the field or along the tile drains, whereas the least productive areas are intermediate in position. These better yields in the favorable seasons and on the more productive areas may be due to a condition favorable for maximum crop utilization of potassium or to a condition that limits the accumulation of a toxic substance, such as an excess of nitrates. In order to determine which of these conditions obtains, the response of an alkali soil to potash treatment was studied at different moisture and temperature conditions in the greenhouse. During the season of 1927 the field from which the soil was taken produced 5.75 bushels of corn to the acre without treatment and 60 bushels an acre where muriate of potash was hill-dropped at the rate of 75 pounds an acre.

After reaching the greenhouse, the soil was leached with distilled water in order to remove most of the soluble salts, particularly nitrates, dried to a

TABLE 5
Influence of moisture and temperature upon nitrate content of a brown sandy loam from McHenry County

INCUBATION PERIOD	NITRATES IN DISPLACED SOIL SOLUTION					
	Soil moisture content					
	21 per cent			32 per cent		
	Soil temperature					
	16° C.	23° C.	30° C.	16° C.	23° C.	30° C.
days	p p m.	p p m.	p p m.	p p m.	p p m.	p p m.
21	448	533	664	330	425	512
49	535	518	971	510	652	861

moisture content of 21 per cent, then sieved, and thoroughly mixed. Asphalt-painted galvanized containers were filled with equal weights of the soil and then placed in constant-temperature tanks held at 16, 23, and 30° C. respectively. At this time the soil contained 60 p.p.m. of nitrates (NO_3) in the displaced soil solution. The soil in half of the containers at each temperature was held at a moisture content of 21 per cent, while in the other half it was maintained at 32 per cent. The soil was kept under these several moisture and temperature conditions for three weeks, after which it was planted to corn. One series under these same conditions was kept fallow for chemical studies, in order that the trend of nitrate accumulation might be followed. The nitrate content of the fallow soil at the time of planting and harvesting of the corn is shown in table 5.

It is clear that the speed of nitrate accumulation has been greatest in the soils which were kept at a low moisture and at the highest temperature, whereas the least accumulation occurred where the moisture content was high and the temperature low.

The response of the corn crop under these moisture and temperature conditions to an application of potassium chloride at the rate of 450 pounds an acre is recorded in table 6.

It is apparent that moisture and temperature influenced the responsiveness of these soils to potash applications. It is of interest to find that the combination of moisture and temperature which resulted in the lowest nitrate content both at the time of planting and harvesting is the one which gave the greatest increase in corn yield for potash fertilization, and that the soil having the highest nitrate content at planting and harvest times gave the smallest re-

TABLE 6

Influence of potassium chloride upon the weight of corn grown on brown sandy loam from McHenry County, at different moisture and temperature conditions

SOIL TEMPERATURE	WEIGHT OF CORN				DIXON SOIL CHECK
	21 per cent moisture		32 per cent moisture		
	None	KCl	None	KCl	
°C.	gm.	gm	gm	gm.	
16	0.54	0.89	0.55	1.13	0.92
23	0.77	1.17	0.78	1.26	1.15
30	1.14	1.41	1.08	1.38	1.85

TABLE 7

Corn yields on black sandy loam in Cass County

NO. PLOTS IN AVERAGE	SOIL TREATMENT		CORN YIELDS	INCREASE OVER CHECK
	Kind	Rate per acre		
		lbs.	bu.	per cent
6	None	28.5
4	Straw	6,000	43.9	54.0
3	KCl	150	69.1	142.4
3	KCl	200	66.9	134.7
4	{ KCl	150 to 200	69.0	142.1
	{ Straw	6,000		

sponse to the application of potash salts. It may be concluded that the conditions which affect the rate of nitrate accumulation also influences the seasonal response of crops on these soils. Therefore, comparatively poor corn yields may be expected in seasons which favor the accumulation of nitrates.

FIELD EXPERIMENTS

Cass County experiment

In order to be of the greatest value, greenhouse trials should be supplemented by field experiments. For that reason, field studies were conducted on four

soils in four counties of the state. It seemed desirable in the beginning to study in the field a soil that had been used in the greenhouse. Consequently, the first field experiment was conducted on the black sandy loam soil in Cass County from which the greenhouse soil had previously been obtained. A summary of the results is given in table 7.

Farm practice, particularly cultivation, made it impossible to use the straw as a mulch, and hence it was necessary to plow it under. The smaller response in the field is attributed to this difference in method of application. The results are comparable, however, to the greenhouse results reported in table 2, where the straw was incorporated in the soil. The difference in response brought about by the method of handling straw may be understood when consideration is given to the fact that two factors are responsible for the increased yields. When enough straw to give an adequate supply of potassium for crop needs is plowed under, the soil microorganisms are encouraged to such an extent that they compete with the corn crop for nitrates. This is shown by

TABLE 8
Alfalfa yields on black mixed loam in Winnebago County

LOT NO.	SOIL TREATMENT		YIELD PER ACRE	INCREASE OVER CHECK	
	Kind	Rate per acre			
		<i>lbs.</i>	<i>lbs.</i>	<i>lbs.</i>	<i>per cent</i>
1	Muriate of potash	160	1,114	536	92.7
2	Straw	4,000	1,213	635	109.9
3	Straw	4,000	1,241	663	114.7
	Muriate of potash	160			
4	None		578
5	Muriate of potash	200	1,114	536	92.7

the nitrate nitrogen content of the soil on July 15, at which time the soil without straw contained 201 pounds an acre in the surface soil and 211 pounds in the subsurface, whereas the land with straw contained no determinable amount in the surface and only 22.5 pounds to the acre of nitrate nitrogen in the subsurface. When applied as a mulch, however, the amount of organic matter made available in proportion to the potassium leached into the soil is relatively smaller. Consequently a favorable effect is produced.

The muriate of potash broadcast at rates of 150 and 200 pounds an acre immediately after the corn was planted gave equally good results at both rates.

Winnebago County experiment

In spite of the fact that alfalfa makes a better growth than corn on untreated alkali soil, definite increases in the yields of alfalfa hay have been obtained in Winnebago County from the applications of the same kinds of fertilizer materials which have proved effective on alkali soils of the state. The yields of alfalfa obtained from one cutting in 1926 are given in table 8.

The value of straw and potassium-containing salts for the improvement of alkali soil is again demonstrated by this field experiment. Although less than one-half as much potassium is applied in the straw as is added in the form of potash salts, the gain is apparently somewhat greater.

McHenry County experiment

A field experiment conducted in 1927 on a brown sandy loam on sand in McHenry County gave results which are similar to those obtained on the two fields previously discussed. A summary of the results is given in table 9.

It is obvious that all treatments except the superphosphate have had a favorable influence upon the yield. The large difference in favor of the hill-dropped muriate of potash at the low rate used was somewhat unexpected. Laboratory studies, which are reported later, indicate that this difference in effectiveness is due to the great capacity for the absorption of salts exhibited by this soil.

TABLE 9
Corn yields on brown sandy loam on sand in McHenry County

NO. OF PLOTS	SOIL TREATMENT		CORN YIELDS PER ACRE	INCREASE PER ACRE
	Kind	Rate per acre		
		<i>lbs.</i>	<i>bu.</i>	<i>bu.</i>
6	None		5.75
1	KCl*	75	60.00	54.25
2	Straw	6,000	24.80	19.05
2	Superphosphate	320	2.50	-3.25
3	KCl†	160	22.30	16.55
2	Cow manure	16,000	40.00	34.25
2	Horse manure	16,000	68.50	62.75

* Hill-dropped.

† Broadcast.

In order to apply the fertilizer broadcast at the same rate per unit area of soil as it is applied to the small area surrounding the hill by hill-dropping, several thousand pounds an acre would be required. Consequently, where the fertilizer is hill-dropped, available potassium remains for the nutrition of the corn plant after the absorptive capacity of the soil is satisfied. This condition is not attained with the broadcast distribution and as a result much better yields result from hill-dropping 75 pounds of potash salts than are obtained by broadcasting the material at the rate of 160 pounds an acre.

For the reasons mentioned, the straw treatment has been less effective than the hill-dropped potash salts and practically as effective as the broadcast fertilizer. Unfortunately, weather conditions prevented the use of straw as a mulch, as had originally been planned. Reasons have already been presented for the belief that such a method would have been more effective than plowing under the straw.

The importance of the physiological balance in soils is illustrated by a comparison of the yields from horse and cow manures. Both manures supplied available potassium but only the horse manure had the tendency to lower nitrate accumulation. This fact is well shown by Allison (1) who found that the unfavorable effect of manure on soils containing meager amounts of available nitrogen was due to the stimulation of biological activities with the resulting nitrate assimilation. In such soils where a lack of available nitrogen was a limiting factor, the cow manure was more than 100 per cent better than the horse manure for the first crop. For the same reason, the horse manure is 70 per cent more effective on soil in McHenry County where an excess of nitrates is a limiting factor.

The pronounced effect on crop growth resulting from the application of the horse manure is shown in plate 1.

TABLE 10
Composition of four types of snail shells found in Illinois alkali soils

SAMPLE		INSOLUBLE MATTER	CALCIUM	CALCIUM CARBONATE		MAGNE- SIUM CAR- BONATE BY DIFFER- ENCE
No.	Name*			Calculated from total Ca	Equivalent by titration	
		per cent	per cent	per cent	per cent	per cent
1	<i>Polygyra monodon</i> (Rackett)	1.92	36.4	91.0	96.8	4.87
2	<i>Helisoma trivialis</i> (Say)	0.99	37.6	94.0	97.4	2.86
3	<i>Physa gyrina</i> (Say)	1.41	37.2	93.0	97.5	3.78
4	<i>Skagnicola umbrosa</i> (Say)	0.68	36.5	91.25	92.9	1.39

* Identified by Prof. F. C. Baker.

LABORATORY INVESTIGATIONS

Both greenhouse and field experiments showed the importance of two factors in limiting the yield of crops on alkali soils. These points have been stressed in the discussion of the tests made and even though the evidence seemed conclusive, it appeared desirable to establish the correctness or fallacy of these conclusions by laboratory investigations.

Composition of snail shells

Previous investigators had attributed the toxicity of these soils to the presence of magnesium carbonate, although they state that considerable amounts of calcium carbonate accompany this constituent. Since shells are so common in these soils, a determination of the calcium and magnesium content of shells which appear to be typical seemed worth while. Table 10 gives the comparison of four representative lots.

In the light of the more recent information upon the subject of the calcium/magnesium ratio in its relation to crop production, these data lend little support to the belief that magnesium toxicity is a factor contributing to the unpro-

ductiveness of these soils, especially if the shells furnish any considerable part of the total amount of these elements contained in the soil.

Composition of three alkali soils

Further data bearing on this relationship are given in table 11, which shows the percentage of nitrogen, calcium, magnesium, and potassium in three alkali soils.

The ratio of calcium to magnesium varies from 2.7:1 to 15:1, ratios which contain considerably more calcium than the proponents of the calcium/magnesium ratio theory considered necessary to prevent the toxicity of magnesium carbonate.

These analytical data are also in accord with the statement made in the introduction that these soils are high in nitrogen and usually alkaline in reaction. The deep peat soil from Cass County is an exception to the latter rule, but it is unusually high in nitrogen and somewhat low in potassium. Consequently, it gives striking increases in crop yield when either straw or potash

TABLE 11
Chemical composition of three Illinois alkali soils

COUNTY	NITROGEN	CALCIUM	MAGNE- SIUM	POTASSIUM	REACTION
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>pH</i>
McHenry.....	0.398	0.85	0.320	0.774	7.30
Cass I.....	1.790	1.45	0.301	0.755	5.45
Cass II.....	0.465	5.45	0.346	0.839	8.35

salts are applied (table 3). None of these soils can be said to be actually deficient in total potassium, however, for they contain, on the average, one-half as much of this element as the usual productive corn-belt soils. Apparently their responsiveness to potash treatment is due to a condition causing a low availability, or to a factor increasing the need of available potassium, or to both. Analyses of other soils of a similar character have given results much like those just discussed.

Availability of soil potassium

McIntire (14), Plummer (15), Ehrenberg (8), and others have called attention to the fact that the availability of soil potassium is lowered by the presence of calcium carbonate. Ehrenberg has gone so far as to formulate a lime-potash law and warns against the application of large quantities of limestone. He states that any unfavorable condition resulting from liming may be counteracted by side-dressings of potash salts. Since the alkali soils in Illinois are well supplied with carbonates, it appeared quite probable that the low crop yields resulted at least in part from a lack of available potassium. Conse-

quently, tests were made to determine whether this was the case. Of the methods designed for the estimation of availability, two were used; namely, determinations of the potassium concentration in the displaced soil solution (4), and the determination of potassium replaced by a neutral salt. These two methods, which are assumed to show immediate and potential availability, should give results which are indicative of the condition of the soil potash so far as its usefulness for crop growth is concerned.

Soils ordinarily contain from 20 to 100 p.p.m. of potassium in the displaced solution. Burd (3) found that three sandy loam soils which he studied contained 26, 49, and 78 p.p.m. respectively. In contrast to this, the displaced solution from the McHenry County soil contained less than 1 p.p.m. of potassium. It is apparent, therefore, that but little potassium exists in this soil in a readily soluble form. Similar results were obtained with other alkali soils and in no case were there more than 8 p.p.m. of potassium found in the displaced solution, and that in one of the more productive of the alkali soils.

Evidence of low availability was found also in the small quantity of replaceable potassium in the McHenry County soil. Only 46 p.p.m. was found in this soil, whereas Vandecaveye (17) reports the presence of 182 p.p.m. of replaceable potassium in untreated Carrington loam in Iowa. Unpublished results from the Ohio station indicate a somewhat similar amount of replaceable potassium in productive soils in that state. Since this McHenry County soil is very low in both water-soluble and replaceable potassium, it is not surprising that it is so highly responsive to fertilizers containing this essential element.

Absorption of potash salts

Although the preceding observations explain the responsiveness of these soils to potash fertilization, they do not furnish the reason for the greater effectiveness of hill-dropped salts, particularly since the acre-rate of application was less than one-half as high as in the broadcast method. There yet remains the possibility that the same condition contributing to the low availability of the native soil potassium may also render applied potash salts unavailable.

In order to determine whether this situation obtained and to ascertain what factors were involved if it did exist, five 4-kilo samples of the soil were used in an experiment in which four different amounts of potassium chloride were added to four of the samples, the fifth sample being retained as a check. After thorough mixing, the soils were maintained at approximately 60 per cent of their water-holding capacity. Three samples of each of the five soils were then used for the following determinations:

Sample 1 (a) Water-soluble potassium not fixed by the soil.

(b) Water-soluble calcium replaced by the potassium chloride.

Sample 2 (a) Potassium exchangeable with 0.1 N BaCl₂.

Sample 3 (a) Potassium content of the soil solution.

The first sample remained for one week in the moist condition and then was leached with distilled water until the percolate was free from soluble calcium. The potassium and calcium contents of the leachings were then determined and the amount of potassium converted into a non-leachable form was ascertained by difference.

Obviously this soil has a great capacity for the absorption of salts because less than 3 per cent of the potassium applied as the chloride remained in a soluble form, even when the rate of application was as high as 600 pounds an acre (table 12). Apparently the potash salt is so completely absorbed when broadcast at the rate of 160 pounds an acre that little remains in a soluble form for the nutrition of the plant.

These data also explain another observed phenomenon, namely, the negligible residual effect of potash salts on these soils, which necessitates repeated applications. This fact has been explained ordinarily by the statement that any residual fertilizer material is largely leached out between cropping seasons.

TABLE 12
Absorption of potassium chloride by brown sandy loam from McHenry County

TREATMENT NO.	RATE OF APPLICATION		POTASSIUM LEACHED		AMOUNT OF ADDED K	
	K per kgm.	KCl per acre	Total	Added	Recovered	Absorbed
	gm.	lbs.	gm.	gm.	per cent	per cent
0	0.004
1	0.0394	150	0.0051	0.0011	2.79	97.21
2	0.0788	300	0.0051	0.0011	1.40	98.60
3	0.1576	600	0.0084	0.0044	2.79	97.21
4	0.3152	1,200	0.0296	0.0256	8.12	91.88

This appeared reasonable since many of the soils are sandy loams. It now appears more likely that repeated applications are necessary on account of fixation rather than leaching.

Whenever a soluble salt is added to a soil, an equilibrium is established between the added ions and the soil constituents. Recent investigations by Hissink (10), Gedroiz (9), Kelly (12), and other workers have shown that the colloidal portions of the soil have an important rôle in this reaction. According to their findings, the potassium ion would be expected to replace other positive ions in the colloidal complex and could in turn be replaced by some other ions, and, in this replaceable condition, the potassium is assumed to be potentially available.

Since both field and laboratory investigations gave evidence that added potash salts are changed into unavailable forms, it seemed desirable to determine what proportion had been absorbed as a part of the colloidal complex. Sample 2 was used for this purpose, BaCl_2 being used as the displacing agent (5). The amounts of water-soluble calcium released by the potassium chloride

applications as determined in sample 1 are compared with the exchangeable potassium found from an analysis of sample 2.

These data indicate that from 50 to 70 per cent of the potassium applied as chloride has been used in releasing calcium ions, the amount depending upon the concentration of chloride used, and that with the exception of the largest application, not only a large total amount of calcium was released with the increasing rate of application but also an amount greater in proportion to the potassium added. On the other hand, excepting the highest rate of application, a progressively smaller percentage of potassium is absorbed in a

TABLE 13

Effect of potassium chloride additions upon water-soluble calcium and replaceable potassium in brown sandy loam from McHenry County

TREATMENT NO.	K FIXED BY SOIL*	WATER-SOLUBLE Ca REPLACED BY KCl	ABSORBED K UTILIZED IN Ca REPLACEMENT	K REPLACEABLE BY 0.1N BaCl ₂	
				Absorbed	Total
	gm	gm	per cent	per cent	p.p.m.
0	46.0
1	0.0383	0.0102	51.9	74.0	74.4
2	0.0777	0.0252	63.3	48.0	82.4
3	0.1532	0.0566	72.0	27.4	88.0
4	0.2896	0.0980	66.0	29.3	130.8

* Calculated from columns II and VII of table 12.

TABLE 14

Analyses of displaced solution from brown sandy loam previously treated with potassium chloride at various rates

TREATMENT NO.	K ADDED PER ACRE	K CONTENT OF DISPLACED SOLUTION
	lbs.	p.p.m.
0	0	0.16
1	150	0.20
2	300	0.16
3	600	0.72
4	1,200	3.40

replaceable form as the concentration of applied salts increases. Unlike soils which are neutral or acidic in reaction, here is a condition wherein only small portions of adsorbed salts can be recovered by leaching with other neutral salts.

Composition of displaced soil solution

A third portion of each of the soils receiving the potassium chloride application was used in determining the potassium content of the displaced solution. They were allowed to stand at approximately 60 per cent of their water-holding capacity for 21 days in order to establish a condition of equilibrium. A dis-

placement with water was then made and the solution analyzed with the following results.

A comparison of tables 13 and 14 shows that a close relationship exists between the replaceable potassium and the amount of this element in the displaced solution. It is significant that this soil, on which corn showed comparatively little response to an application of 160 pounds of muriate of potash under field conditions, shows little change in the soil solution with small applications of potassium salts, and that even when applied at a high rate, the amount in the soil solution is much less than most soils contain.

In addition to the low available potassium content, the importance of a high soil nitrate-nitrogen content in limiting crop yields has been mentioned. Table 5 shows that this soil not only produces nitrates, but that they are formed rapidly, going from 60 p. p. m. to 971 p. p. m. in a period of 49 days. In fact, a large part of the soluble salts which appear on the surface of the soil as a whitish deposit is composed of calcium nitrate and sulfate. The nitrate gives to the soil a characteristic brownish appearance, whereas the sulfate is

TABLE 15
Composition of displaced soil solution of three alkali soils

COUNTY	MOISTURE CONTENT	ION CONCENTRATION OF DISPLACED SOLUTION						
		Ca	SO ₄	NO ₃	Mg	K	PO ₄	Al
	per cent	p p m.	p p m.	p p m.	p p m.	p p m.	p p m.	p p m.
McHenry.....	22.0	160.0	48.0	430.0	48.0	0.10	0.04	Trace
McLean.....	31.9	390.0	135.0	910.0	19.0	8.00	0.10	Trace
Rock Island.....	22.8	578.0	1,040.0	1,532.0	0	0.03	Trace

distinctly white. Analyses showed that the displaced solution from two of these soils contained 45 and 53 per cent respectively of the total solids in the form of calcium nitrate, the remainder being composed of a combination of calcium, sulfate, magnesium, and sodium ions with traces of other elements. The distribution of the more important ions in three other typical alkali soils is given in table 15.

It is apparent from these results, which have been confirmed by numerous other analyses, that these soils furnish a displaced solution which is low in potassium and phosphate and correspondingly high in calcium, nitrate, and sulfate ions. It should be stated that the first two ions are found in fairly constant amounts, whereas the three latter vary from time to time, depending upon their removal by leaching, utilization by higher or lower plants, and accumulation as a result of nitrification. The latter statement is based upon the fact that sulfate and calcium solubility is greatly affected by the process of nitrate formation.

GENERAL DISCUSSION OF RESULTS

The laboratory, greenhouse, and field results indicate that the unproductiveness of the alkali soils of Illinois is not due to a single condition but to a combination of factors. Chief among these is a lack of available potassium and a concentration of nitrate nitrogen which is harmful. The toxicity of the nitrate nitrogen is not due solely to the presence of the nitrates, but results from an accumulation of these salts in the absence of available potassium. This assumes that a favorable physiological balance is desirable, particularly with reference to potassium and nitrate nitrogen.

The data presented show that these soils in general are not markedly deficient in total potassium but that they are lacking in an available supply of this element, as judged by the potassium content of the displaced soil solution, by replaceable potassium, by the effect of applied potash salts upon the crop yields, and by the absorption of applied potassium in non-replaceable combination. The investigations of several workers have shown that the availability of this element is lowered by liming, consequently it appears highly probable that the deficiency of usable potassium is the result of the high carbonate content. The fact that the crop yields on an acid peaty soil were unfavorably influenced by a 50-ton application of high calcium limestone further substantiates this idea. Evidence that nitrate accumulations are also to be considered as an unfavorable factor is found in the following observations.:

Applications of straw as a mulch have a much more pronounced benefit upon crop yields than an amount of potash salts containing an equivalent quantity of the element potassium.

The immediate effect of straw is beneficial, whereas sweet clover has little favorable influence, in spite of the fact that it contains potassium. That sweet clover has a positive influence upon nitrate accumulation whereas straw has a negative effect, has been well established by other investigators.

Straw ashes are less beneficial than the unburned straw when it is used as a mulch, whereas the sweet clover ashes are fully as effective as the unburned sweet clover. Such a result is in accord with the idea that the difference between these two materials for improvement of alkali soils depends upon their effect upon nitrate accumulation. Consequently, it is doubtful whether the use of sweet clover can be depended upon for the immediate improvement of these soils, except perhaps where they possess a clay subsoil from which potassium may be extracted in relatively large amounts. Its effectiveness would be limited by the fact that nitrate accumulation induced by the decaying sweet clover would offset to a large extent any favorable influence that might be exerted by the potassium rendered available through the growing and subsequent death of the plant.

Since sweet clover makes a rank growth on many of the alkali soils without potash application, it is apparent that the potassium contained in the soil is not completely unavailable; in fact, Russell (16) states "that clovers have less capacity for absorbing potassium from the soil solution than the grasses." Inasmuch as this crop uses relatively large quantities of nitrogen in proportion to the potassium requirement, it seems probable that these soils would furnish a more favorable environment for this than for other crops requiring more potassium and less nitrogen.

Horse manure is much more valuable than cow manure for improving this kind of land. Other investigators have found that horse manure lowers nitrate accumulations, whereas cow manure tends to increase them. Consequently, the latter is superior for soils having a deficiency of

available nitrogen, particularly for the first crop. It is for the same reason that the horse manure is more effective on these soils than cow manure.

These soils contain large amounts of calcium nitrate, which is easily removed by leaching but which quickly reappears as a result of nitrification.

One Bureau County soil produced more than 600 p.p.m. of nitrate nitrogen as measured in the displaced solution in a period of seven days. Obviously, adequate drainage will not bring about the permanent removal of the soluble salts except as it encourages more rapid decay of the easily oxidizable organic matter. Good drainage is desirable, however, for other reasons and when accompanied by the use of non-nitrogenous organic matter, and the application of potash salts is indispensable for permanent improvement of these lands.

SUMMARY

The unproductivity of numerous areas of land in the northern half of Illinois is caused, not by a single condition, but is the resultant of two factors; namely, low availability of potassium and an excessive amount of nitrate nitrogen.

The low availability of potassium on most of these soils is due to an alkaline reaction rather than to a marked deficiency of this element.

The value of straw for improving the crop-producing capacity of the soils is attributed in part to the potassium which it contains. In addition, the crop is favored by a lowering of the nitrate nitrogen content of the soil resulting from the straw application. This explains the greater efficiency of unburned straw compared to its ashes in increasing yields.

For the same reason, horse manure is more valuable than cow manure on these soils, whereas the opposite condition obtains on soils in which a deficiency of available nitrogen is a limiting factor.

A favorable physiological balance in the soil is desirable, particularly with respect to potassium and nitrate nitrogen.

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PLATE 1

EFFECT OF HORSE MANURE ON CORN CROP GROWN ON A BROWN SANDY LOAM ON SAND IN
McHENRY COUNTY

FIG. 1. Horse manure plowed under at rate of 8 tons an acre previous to planting corn.

FIG. 2. No soil treatment.



FIG. 1



FIG. 2

THE STATE OF UNSATURATION OF THE SOIL IN RELATION TO ITS FIELD BEHAVIOR AND LIME REQUIREMENT

P. E. TURNER

The Imperial College of Tropical Agriculture, Trinidad, B. W. I.

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That a portion of the metallic elements and of the hydrogen present in soils is capable, under suitable circumstances, of passing into the ionic state and entering into double decomposition with the cations of salts has been established by many investigators. The ions, called for this reason "exchangeable ions," found in appreciable quantities in the vast majority of agricultural soils are six; namely, hydrogen, sodium, potassium, ammonium, magnesium, and calcium. Two others, iron and aluminium, are frequently, but not invariably, present. The extent to which ionization can proceed appears limited to a fairly well-defined value, largely dependent on the soil content of inorganic and organic colloidal material. This value, denoted by the term "saturation capacity," varies from soil to soil, but is independent of the proportions in which the various ions occur (8).

The investigations of Gedroiz, Hissink, and others have demonstrated that the numerical relationship which exists between the ions is nevertheless of extreme importance, in that upon it the physico-chemical properties and the field behavior of the soil largely depend. Each positive ion, when associated with the colloidal complex of the soil, appears to form a compound with unique characteristics, and if present in preponderating amounts, impresses its individual peculiarities on the soil as a whole. Sodium and hydrogen ions, for example, accentuate the hydrophilic nature of the colloidal material, thereby emphasising its tendency to exist in a dispersed, deflocculated condition when moist and to compact to a cemented mass when dry (5, 7, 17, 30). Ammonium, potassium, and magnesium ions appear to exert a similar but decreasingly marked effect (7, 30). Calcium, iron, and aluminium ions render the colloidal material, by comparison, hydrophobic, and modify its properties to more desirable proportions (7, 17, 30).

The data which have been accumulated show that under normal circumstances in humid regions the soil content of exchangeable sodium, potassium, magnesium, iron, and aluminium is, as a rule, so small that the ions can exert but a minor influence on soil behavior. The investigations of McLean and Robinson (25) demonstrate that ammonium ions rarely occur in sufficient quantity to possess an appreciable effect. There is abundant evidence that exchangeable calcium usually exists in significant amounts, which may exceed 1 per cent of the total weight of the air-dry soil. Information with regard to the hydrogen ion is less definite and somewhat contradictory. Gedroiz found that although many "base unsaturated" Russian soils contained but small amounts (10), there was present in others the equivalent of 0.50 per cent calcium (9). From the earlier data of Joffe and McLean (21) it appears that in no case were more than 4.5 mgm. E. present in the soils they examined. Correspondingly low values were obtained by Kelley and Brown (24). The researches of Hissink (17), Gehring, Peggau, and Wehrmann (11), Osugi and Sano (28), and others, and the later investigations of Joffe and McLean (22), however, indicate that exchangeable hydrogen normally occurs in relatively considerable extent. The work of Bradfield (3), which suggests that complete saturation of clays with bases occurs only when a pH value of 10 or 11 is attained, lends indirect support to this view. If such is the case, hydrogen and calcium

together account for by far the greater majority of the exchangeable ions of the soils of humid climates, and upon the relative proportions of these two ions, and possibly also upon their absolute amounts, the properties of the soils will mainly depend.

The significance of the ratio of hydrogen ions to calcium ions has been realized by many workers, but comparatively few adequate attempts have been made to correlate it with either the behavior of the soil or its need for lime, probably because of the difficulties which underlie the accurate determination of the exchangeable hydrogen. To these difficulties the confusion which exists with regard to the soil content of ionizable hydrogen appears mainly to be due.

Hissink (16) at first avoided them by expressing the calcium ion (together with the other metallic ions) as a ratio of the clay and very fine silt, which he assumed to be a measure of the soil colloidal material and therefore of its saturation capacity. By means of such ratio values, soils in poor and in good condition can be distinguished, even though they differ widely in type and capacity for ion exchange (35). This method, however, is not sufficiently precise to reflect the gradation in soil properties caused by a relatively small change in ion content, unless the soils are otherwise very uniform in character. Later Hissink (17) determined the replaceable hydrogen by measuring the distribution of barium ions between varying amounts of decinormal barium hydroxide solution and a constant weight of soil. The results obtained were approximate, but the ratios they bore to the sum of the equivalents of the exchangeable ions present enabled him to differentiate between new, medium, and old soils of Holland.

The second method of Hissink has been subjected to much criticism on the following and other (34) grounds: (a) that side reactions may occur between the barium hydroxide solution and the soil, and that, consequently, an overestimation is made of the replaceable hydrogen present (24, 32); and (b) that alkaline soils of pH 9.2 to 10.2 give results by his titration process which indicate the occurrence of considerable amounts of replaceable hydrogen, whereas by his alternative conductometric method the same soils appear to be fully saturated with bases (32).

Gehring, Peggau, and Wehrmann (11) have shown that the ratio of exchangeable calcium to saturation capacity is related to the lime requirement of the soil and to the increase in crop yield following requisite liming. They determined saturation capacity by treating the soil with a saturated solution of calcium hydroxide, followed by heating to 60°C. After standing for 24 hours the soil suspension was saturated with carbon dioxide to precipitate the excess calcium hydroxide, and thoroughly boiled to decompose any calcium bicarbonate formed. The soil was then leached to two liters with normal sodium chloride solution, and its content of exchangeable calcium determined according to the method of Hissink. This procedure is open to objection because of the somewhat drastic treatment to which the soil is exposed. The inorganic and organic colloidal components of the soil are extremely reactive in character, and are readily subject to decomposition. Thorough boiling, therefore, may affect appreciably their capacity for ion exchange.

The ideas underlying the investigations of Hissink and of Gehring, Peggau and Wehrmann, in view of their extreme practical importance, merit wider application, and have been extended to the examination of soils of Trinidad. In view, however, of the objections made to their technique, alternative methods have been used. The work has been confined to the measurement of the ionizable hydrogen and calcium present in the soil, but a critical study has been made of the measures adopted, in order, if possible, to place the experimental procedure on a more firmly established basis.

PART I.—MEASUREMENT OF THE STATE OF UNSATURATION

DETERMINATION OF EXCHANGEABLE HYDROGEN AND CALCIUM

Joffe and McLean (21) and others have measured the exchangeable hydrogen present in soils by leaching them with neutral salt solution until the filtrates were no longer acid or contained no traces of calcium, and by titrating the extracts. Exact results are not given by such methods, for they involve the titration of small amounts of hydrogen ion in large volumes of solution. Page and Williams (29) overcame this difficulty by mixing the soil with calcium carbonate prior to leaching with sodium chloride solution. The hydrogen ions removed then interact with the carbonate, and an equivalent amount of calcium, which can be measured with precision, passes into solution. The method of Page and Williams, with some modifications, has been used in the present investigations.

Standard procedure adopted

The soils were air-dried and representative 25-gm. samples were intimately mixed with calcium carbonate, well moistened, treated with 100 cc. hot N NaCl, allowed to stand for some days, and then leached to two liters with this reagent. After the necessary correction was made for the calcium carbonate dissolved by, and originally present in, the leaching agent (0.038 gm., when expressed as calcium oxide, to the liter.), the total calcium passing into solution in the two liters was taken as a measure of the sum of the exchangeable hydrogen and calcium present in the soil sample. A discussion and fuller description of the method have been given in a previous paper (34).

The value thus obtained is an approximation to the total capacity of the soil for ion exchange, and for convenience is in future referred to as the saturation capacity (T). The soil content of exchangeable calcium (S) was separately estimated by the standard method of Hissink (16), and the replaced hydrogen, or, alternatively, the saturation deficit of the soil for calcium ions ($T-S$), was determined by difference. The ratio of exchangeable hydrogen to exchangeable calcium is expressed indirectly by means of the quotient $\frac{100 (T-S)}{T}$. This

ratio is termed, in accordance with current usage, "the degree of unsaturation of the soil with respect to calcium ions."

The procedure described for the determination of the exchangeable hydrogen and calcium together appears free from a main defect of the second method of Hissink (17) in that hydroxyl ions in high concentration are not brought into contact with the soil. It does not possess the disadvantage which characterises the technique of Bobko and Askinasi (2), Kelley and Brown (24), and Joffe and McLean (22). These workers replaced the ions originally present in the soil by those of one kind, removing all traces of the salt solution used by leaching with water. The protracted washing necessary may cause loss of the

replacing ion through solution of humates and hydrolysis. Further, it appears preferable to the method of Gehring, Peggau, and Wehrmann (11) in that, except for the initial use of hot sodium chloride, which can have but little heating effect upon the soil particles, the whole process is performed at normal temperature.

RELIABILITY OF THE METHOD

Effect of errors due to subsampling and technique

With methods of the type adopted it appears advisable to determine the extent to which the data obtained for the particular subsample examined are representative of the bulk sample from which it is taken, and also the extent to which they are affected by such deviations from a constant technique as may occur. Both possibilities of error are of particular importance in the determination of saturation deficit, which is not only measured by difference, but frequently possesses a small value. An endeavor therefore has been made to find the magnitude of the variations which may occur in the processes used. Three soils, each belonging to a different type, all of high saturation capacity, but containing approximately 0.1, 0.4, and 0.9 per cent exchangeable calcium, respectively, were selected for the purpose.

From the bulk samples of each of these soils six representative samples were obtained by the normal method. Three were leached to one liter for the determination of exchangeable calcium, and three, after the necessary preliminary treatment, were leached to two liters according to the standard procedure adopted for the measurement of saturation capacity. By determination of the calcium present in aliquot portions of each of the filtrates, two separate estimates (*a* and *b*, table 1) of the total calcium present in each liter were obtained. The estimation procedure followed was identical with that normally used in routine investigations. The data obtained for each soil were pooled (6) and the net standard deviations due to technique and subsampling, and the observed standard deviations, of the values obtained for its content of exchangeable calcium, its saturation capacity, saturation deficit, and degree of unsaturation were calculated. They are recorded in table 1.

The net standard deviations due to errors in technique of the values obtained for the contents of exchangeable calcium and the saturation capacities of the three soils examined are so small that they are negligible. They afford an excellent demonstration of the precision with which measurements of exchangeable calcium can be made by the method of Hissink (16) and of exchangeable calcium and hydrogen, together, by the modified method of Page and Williams, whether the quantities estimated are large or small. The net standard deviations due to errors in subsampling in the determination of exchangeable calcium (0.001 per cent CaO) are also very small, but in the case of saturation capacity they may attain a value of 0.012 per cent CaO. In both

determinations the errors due to subsampling are much larger than the errors due to technique, indicating that a closer estimate of the contents of exchangeable calcium and of saturation capacities of bulk samples could be obtained, with the same number of analytical measurements, by ignoring the errors due

TABLE 1

Errors due to subsampling and technique in determination of exchangeable calcium, saturation capacity, saturation deficit, and degree of unsaturation

SOIL TYPE*	DETERMINATION	SUBSAMPLE			MEAN OF ALL	NET STANDARD DEVIATION DUE TO		OBSERVED STANDARD DEVIATION	OBSERVED S.D. AS PER CENT OF MEAN	
		(I)	(II)	(III)		Sub-sampling	Technique			
		per cent	per cent	per cent	per cent	per cent	per cent	per cent		
G	Exchangeable calcium	(a)	0.112	0.109	0.111	0.001	0.0003	0.001	1.2
		(b)	0.112	0.110	0.111				
		Mean	0.112	0.110	0.111	0.111				
	Saturation capacity	(a)	0.906	0.929	0.924	0.012	0.0003	0.012	1.3
		(b)	0.906	0.929	0.925				
		Mean	0.906	0.929	0.925	0.920				
	Saturation deficit	0.809	0.012	1.5	
Degree of unsaturation	87.9	1.77	2.0		
C	Exchangeable calcium	(a)	0.374	0.371	0.374	0.001	0.0006	0.001	0.28
		(b)	0.374	0.373	0.373				
		Mean	0.374	0.372	0.374	0.373				
	Saturation capacity	(a)	0.835	0.836	0.838	0.001	0.0000	0.0015	0.18
		(b)	0.835	0.836	0.838				
		Mean	0.835	0.836	0.838	0.836				
	Saturation deficit	0.463	0.002	0.39	
Degree of unsaturation	55.4	0.243	0.44		
D	Exchangeable calcium	(a)	0.907	0.910	0.908	0.001	0.0003	0.001	0.14
		(b)	0.907	0.909	0.908				
		Mean	0.907	0.910	0.908	0.908				
	Saturation capacity	(a)	1.160	1.166	1.150	0.009	0.001	0.010	0.82
		(b)	1.160	1.170	1.148				
		Mean	1.160	1.168	1.149	1.159				
	Saturation deficit	0.251	0.010	3.82	
Degree of unsaturation	20.0	0.85	4.25		

* A description of these soil types is given later in this paper.

to technique and making single analyses of the filtrates from double the number of subsamples. The observed standard deviations of the values for exchangeable calcium and saturation capacity in no case exceed 1.5 per cent when measured as percentages of the means.

The observed standard deviation recorded in table 1 for the saturation deficit

of each soil was calculated indirectly from the corresponding deviations for exchangeable calcium and saturation capacity. That for the degree of unsaturation was similarly obtained from the observed standard deviations of the saturation deficit and saturation capacity. The small variations which occur in the values for exchangeable calcium render the observed standard deviations for the saturation deficit but little larger than those for the corresponding saturation capacities. Neither the observed deviations for the saturation deficit nor the degree of unsaturation attain a value equal to 5 per cent of the mean, even when the saturation deficit and degree of unsaturation are as small as 0.25 per cent CaO and 20 per cent, respectively.

It appears from the data in table 1, therefore, that the mean values obtained by the standard procedure used, for exchangeable calcium, saturation capacity, saturation deficit, and degree of unsaturation, from duplicate estimations on a single subsample, give a sufficiently close approximation to those which would be obtained by the examination of a number of subsamples by this procedure, for most purposes of soil differentiation and characterization, but that when the significance of small differences is in question, three or more subsamples should be examined. The measurements obtained from a single subsample appear adequate for the investigations later described in this paper.

Effect of continued leaching on the values obtained

It is questionable whether leaching processes, as a class, displace from the soil all the hydrogen which can pass into the ionic form. Evidence of incomplete replacement by the modified method of Page and Williams has already been obtained (34). Further indications to the same effect are given by the pH values of the sodium clays¹ formed by the use of this method in determining the saturation capacities of the soils described in Part III of this paper. These values are recorded in column 4 of tables 3, 4, and 5. All of them lie between pH 7.70 and pH 8.37, a range which is much less alkaline in reaction than that obtained by Bradfield (3) for base-saturated soils. For these reasons an estimation has been made of the relative extent to which replacement of the ionizable hydrogen is limited by restricting the leaching process to two liters, and of the effect which partial replacement may exert upon the values for saturation capacity, saturation deficit, and degree of unsaturation when used for the purpose of soil differentiation and characterization.

Three soils, of high, medium, and low ionic capacity, respectively, the exchangeable calcium content of which had previously been determined, were selected from the various types later examined, and were leached to five liters with normal sodium chloride. With these soils certain modifications were introduced into the procedure used. After the soils were mixed with

¹ Previous to the determination of their pH values, these clays were not washed free from the sodium chloride solution used in their preparation. The sodium chloride present may repress the ionization of the sodium clays, thereby reducing their pH values.

calcium carbonate, treated with 100 cc. of hot salt solution, and allowed to stand, they were stirred, left to settle, and decanted 10 times with 50 cc. of cold salt solution previous to filtration. Instead of allowing them to remain in the funnels throughout the leaching process, they were removed to beakers after each separate liter had run through, stirred, and decanted 10 times as before, and then retransferred to funnels. The preliminary treatments of each soil, the decanting and leaching processes, and the subsequent analytical operations were performed side by side, in order that the data obtained for these soils should be strictly comparable. The calcium present in each liter

TABLE 2

Effect of continued leaching in determination of saturation capacity, saturation deficit, and degree of unsaturation

(a) *Saturation capacity and saturation deficit*

SOIL TYPE*	EX-CHANGE-ABLE Ca	AMOUNT EXTRACTED BY					SATURATION CAPACITY		SATURATION DEFICIT		RATIO VALUES	
		Liter I	Liter II	Liter III	Liter IV	Liter V	Calculated from				$\frac{T_2}{T_1}$	$\frac{T_2-S}{T_1-S}$
							1st 21 (T ₂)	1st 51 (T ₅)	1st 21 (T ₂ -S)	1st 51 (T ₅ -S)		
Gm. CaO per 100 gm air-dry soil												
F	0.069	0.141	0.024	0.014	0.008	0.001	0.165	0.188	0.096	0.119	0.88	0.81
G	0.222	0.596	0.118	0.064	0.044	0.044	0.714	0.866	0.492	0.644	0.82	0.76
B	0.315	0.732	0.122	0.080	0.062	0.058	0.854	1.054	0.539	0.739	0.81	0.73

(b) *Degree of unsaturation*

SOIL TYPE	DEGREE OF UNSATURATION (PER CENT) CALCULATED FROM					RATIO VALUES			
	Liter I U ₁	1st 21 U ₂	1st 31 U ₃	1st 41 U ₄	1st 51 U ₅	$\frac{U_1}{U_5}$	$\frac{U_2}{U_5}$	$\frac{U_3}{U_5}$	$\frac{U_4}{U_5}$
F	51.1	58.2	61.5	63.1	63.3	0.81	0.92	0.97	1.00
G	62.8	68.9	71.5	73.0	74.4	0.84	0.93	0.96	0.98
B	57.0	63.1	66.3	68.4	70.1	0.81	0.90	0.95	0.98

* A description of these soil types is given later in this paper.

of filtrate was determined in duplicate. As the amount of calcium carbonate dissolved was found to be unaltered by the modifications introduced, the customary adjustments were made, for each of the five liters, for its solubility in the leaching agent. The corrected mean values obtained are given in table 2. To avoid confusion the amounts of calcium passing into solution through exchange reactions with the soil in two liters and five liters are for the time being termed the "apparent" and "proximate" saturation capacities, respectively. The corresponding saturation deficits and degrees of saturation are similarly designated.

The data in table 2 show that far larger amounts of hydrogen are replaced

by the first liter of filtrate than by each subsequent liter; that fairly large quantities are extracted by the second liter; and that significant amounts may be removed by the third, fourth, and fifth liters. The amounts displaced by liters III, IV and V from the soil of low saturation capacity (0.014, 0.008, and 0.001 per cent CaO respectively,) are small. They demonstrate that ion exchange in this soil is, for practical purposes, complete when five liters of filtrate have been obtained; and they confirm, to some extent, the validity of the correction used for the solubility of calcium carbonate in the leaching agent. The quantities present in liters III, IV, and V together, become increasingly important, however, as the degree of colloidality of the soil increases. They amount to 0.15 per cent CaO for the soil of medium saturation capacity and 0.20 per cent CaO for the soil of highest saturation capacity. Such data suggest that the greater the capacity of the soil for ion exchange, the greater is the volume of filtrate necessary completely to remove the ionizable hydrogen. As the quantities present in liters IV and V of the filtrate from both the heavier soils differ but little in magnitude, leaching to an indefinite volume appears to be required for this purpose.

The pH values of the sodium clays formed by leaching the three soils of types F, G, and B to five liters were found to be 8.37, 8.25, and 8.28, respectively. In spite of the continued leaching, these values are much lower than those obtained by Bradfield (3) for base-saturated soils. It is perhaps noteworthy that Osugi and Sano (28) found the soils they examined to be saturated at values ranging from pH 8.6 to 11.

DISCUSSION

It is clear from the data accumulated that the replacement resulting from leaching to two liters by the modified method of Page and Williams can be only partial in character. Complete replacement, however, need not necessarily be essential for the object of soil differentiation and characterization, provided that for each soil: (a) constant results for the amount of calcium and hydrogen replaced are obtained on repetition; (b) the ratios of the apparent saturation capacity, saturation deficit, and degree of unsaturation to their respective true values are independent (i) of the magnitude of the saturation capacity of the soil and (ii) of the nature of the colloidal material of the type from which the soil is drawn; and (c) exchange of hydrogen occurs to such an extent that the ratios of the replaced hydrogen to calcium in good and poor soils are sufficiently different adequately to reflect the dissimilarity in their properties.

The data given in table 1 demonstrate that the first of these stipulations is satisfied, in that the measurements of saturation capacity and saturation deficit obtained on repetition are in close accordance.

There are given in table 2 *a* the apparent and proximate saturation capacities (denoted by T_2 and T_6 , respectively,) and saturation deficits [$(T_2 - S)$ and $(T_6 - S)$] of the three soils leached to five liters. From them the ratio

values $\frac{T_2}{T_6}$ and $\frac{T_2 - S}{T_6 - S}$ have been calculated. The data obtained demonstrate that the ratio of the apparent to the proximate saturation capacity $\frac{T_2}{T_6}$ varies from 0.88 for the soil of lowest saturation capacity to 0.81 for the soil of highest saturation capacity. The difference between these values is smaller than that between the ratios of the apparent to the true saturation capacities for these soils, for, as table 2 indicates, further leaching would remove greater quantities of ion from the soil of high than of low saturation capacity. Assuming that the standard deviation of the values obtained for the apparent and proximate saturation capacities are in each case equal to 1.3 per cent of their means (the maximum deviation found for the saturation capacities recorded in table 1), the standard error of the difference between the ratio values 0.88 and 0.81 is 0.022. It appears, therefore, that the percentage of the sum equivalent of the calcium and hydrogen ions present, which are removed by the first two liters of filtrate, decreases significantly as the saturation capacity increases in magnitude.

The pH values of the sodium clays recorded in tables 3, 4, and 5 tend to confirm this conclusion, in that they exhibit a fairly well-defined propensity to decrease with increasing saturation capacity. The correlation coefficient of the pH values of these clays with their saturation capacities, for the 49 soils for which both sets of data have been obtained, is -0.490 , $P = < 0.01$. The corresponding correlation coefficient for the 15 soils of type G is -0.548 , $P = < 0.05$.

A similar state of affairs holds for the measurements of saturation deficit. Eighty-one per cent of the hydrogen ions extracted by five liters of filtrate are present in the first two liters from the soil of lowest saturation capacity, 76 per cent from the soil of medium saturation capacity, and only 73 per cent from the soil of highest saturation capacity. If the standard deviations of the values for the saturation deficits of the soils of minimum and maximum saturation capacity are taken in this case as equal to 2 per cent of their respective means, the standard error of the difference of their ratio values amounts to 3.1 per cent. The difference of 8 per cent is significant under such circumstances. As before, this difference would be greater if the measurements for the proximate saturation deficits were replaced by those for the true saturation deficits.

This lack of consistency in the values of the ratios $\frac{T_2}{T_6}$ and $\frac{(T_2 - S)}{(T_6 - S)}$ may be attributed to differences in the nature of the colloidal material of the soils examined. Another explanation is that the leaching solution comes into equilibrium with the soil solution more quickly with soils of low than of high saturation capacity, and that, although the soil is transferred to a beaker after each liter has run through, and thoroughly stirred and decanted 10 times before leaching

is continued, equilibrium conditions are not attained with soils of higher saturation capacity. If these are the contributory causes, there can be little doubt that in the determination of saturation capacity and saturation deficit the leaching process should be continued until as large a volume of filtrate as practicable is obtained.

TABLE 3
Lime status and physical constants of soils in good condition (Group I)

1	2	3	4	5	6	7		8		9		10	11	
SOIL TYPE	SOIL NUMBER	pH VALUE	pH VALUE OF Na CLAY	C. + F.S.*II	CaCO ₃	SATURATION CAPACITY (T)		EXCHANGE-ABLE Ca (S)		SATURATION DEFICIT (T-S)		DEGREE OF UNSATURATION [100(T-S)/T]	C. + F.S.*II	
				per cent		per cent	gm. CaO	mgm. E.	gm. CaO	mgm. E.	gm. CaO			mgm. E.
Naparima District														
C	M.	64	7.14	7.81	45.32	0.28	0.588	21.00	0.470	16.80	0.118	4.20	20.1	0.37
	M.	101	7.51	8.01	58.53	Trace	0.700	25.00	0.547	19.54	0.153	5.46	21.9	0.33
D	M.	181	7.22	7.90	62.38	0.05	1.087	38.82	0.935	33.39	0.152	5.43	14.0	0.54
	M.	40	5.69	7.86	63.60	0.00	0.996	35.57	0.810	28.93	0.186	6.64	18.7	0.45
E	MPD.	7	7.48	7.90	53.05	0.14	1.150	41.07	0.919	32.82	0.231	8.25	20.1	0.62
	M.	42	5.84	7.87	60.00	Trace	1.717	61.32	1.298	46.36	0.419	14.96	24.4	0.77
Central Plain														
G	CA.	15	6.83	7.86	55.17	Trace	0.572	20.43	0.439	15.68	0.133	4.75	23.2	0.28
	B.	39	6.35	8.16	20.00	0.02	0.548	19.57	0.414	14.79	0.134	4.78	24.5	0.74
	CPD.	1	7.87	8.13	22.03	Trace	0.354	12.64	0.256	9.14	0.098	3.50	27.7	0.41
	CA.	16	7.44	8.04	23.99	Trace	0.390	13.93	0.279	9.96	0.111	3.97	28.5	0.42
H	O.	59	6.91	7.93	36.25	Trace	0.420	15.00	0.369	13.18	0.051	1.82	12.1	0.36
	A.	10	7.66	7.70	18.57	Trace	0.247	8.82	0.205	7.32	0.042	1.50	17.0	0.39
Mean value.....												21.0	

* C = clay; F.S. = fine silt.

A third possibility exists, however, namely, that side and secondary reactions, negligible in extent in soils of low saturation capacity, but appreciable in soils of higher saturation capacity, occur during the later stages of the leaching process. Such reactions could be due to: the hydroxyl ions released by hydrolysis of the sodium clay; decomposition of the sodium clay, which is possibly somewhat unstable; and increased hydration of the components of the colloidal complex through continued contact with an aqueous solution. All would tend to increase the value for the saturation capacity. Of these

alternatives the third appears the most probable. The effect of the first is likely to be very small, for ionization of the sodium clay should be almost completely suppressed by the high concentration of sodium ions in the normal salt solution in contact with the soil.

In the investigations later described, leaching was restricted to two liters, partly to economize in time and material and partly to minimize the effect of possible side and secondary reactions. It is necessary to bear in mind, however, that the values thus obtained for saturation capacity and saturation deficit may not be strictly comparable.

TABLE 4
Lime status and physical constants of transition soils (Group II)

1	2	3	4	5	6	7		8		9		10	11
SOIL TYPE	SOIL NUMBER	pH VALUE	pH VALUE OF Na CLAY	C + F.S.*II	CaCO ₃	SATURATION CAPACITY (T)		EXCHANGE-ABLE Ca (S)		SATURATION DEFICIT (T-S)		DEGREE OF UNSAT-URATION [100(T-S)/T]	C + F.S.*II
				(Per 100 gm. air-dry soil)									
				per cent	per cent	gm CaO	mgm E	gm. CaO	mgm. E	gm CaO	mgm. E		
Naparima District													
B	M. 24	5.31	7.79	...	0.00	0.679	24.25	0.468	16.71	0.211	7.54	30.9	...
	M. 48	6.55	7.83	...	0.00	0.664	23.71	0.433	15.46	0.231	8.25	34.8	...
C	M. 71	6.07	7.92	34.63	0.00	0.553	19.75	0.370	13.21	0.183	6.54	33.1	0.38
D	M. 190	6.39	7.78	67.50	0.00	0.930	33.21	0.622	22.21	0.308	11.00	33.1	0.33
Central Plain													
G	W. 48	6.68	7.97	56.00	0.00	0.698	24.93	0.472	16.86	0.226	8.07	32.4	0.30
H	O. 122	6.89	7.97	29.10	0.00	0.329	11.75	0.230	8.21	0.099	3.54	30.1	0.28
Mean value												32.4	...

*C = clay; F.S. = fine silt.

The degree of unsaturation, as calculated on the basis of a one, two, three, four, and five liter extraction, for the three soils examined is recorded in table 2 b. The five values obtained for each soil are denoted by U_1, U_2, \dots, U_5 , respectively, in this table. The difference between U_1 and U_5 amounts to between 11 and 13 per cent for each of the soils examined. This difference is very substantial, and by comparison with the observed standard deviations of the values for the degrees of unsaturation recorded in table 1, is undoubtedly significant. The two-liter values (U_2) lie in each case approximately midway between the extreme values obtained (U_1 and U_5).

The ratios which U_1, U_2, U_3 , and U_4 bear to U_5 are also given in table 2 b. The three values for $\frac{U_1}{U_5}$ approximate closely a constant 0.82. Those for $\frac{U_2}{U_5}$

TABLE 5

Lime status and physical constants of soils in poor condition (Group III)

1	2	3	4	5	6	7		8		9		10	11		
SOIL TYPE	SOIL NUMBER	pH VALUE	pH VALUE OF Na CLAY	C. + F.S.* II	CaCO ₃	SATURATION CAPACITY (T)		EXCHANGE-ABLE Ca (S)		SATURATION DEFICIT (T-S)		DEGREE OF UNSAT-URATION [100(T-S)/T]	S C. + F.S.* II		
				per cent	per cent	(Per 100 gm. air-dry soil)									
						gm. CaO	mgm. E.	gm. CaO	mgm. E.	gm. CaO	mgm. E.				
Naparima District															
A	MH.	15 5.31	7.70	74.60	0.00	0.737	26.32	0.401	14.32	0.336	12.00	45.6	0.19		
	M.	50 5.04	7.73	62.67	0.00	0.828	29.57	0.431	15.39	0.397	14.18	48.0	0.25		
B	M.	22 5.63	7.88	0.00	0.611	21.82	0.359	12.82	0.252	9.00	41.2		
	M.	25 4.93	7.83	0.00	0.810	28.93	0.460	16.43	0.350	12.50	43.2		
	M.	27 5.79	8.00	0.00	0.579	20.68	0.320	11.43	0.259	9.25	44.7		
	M.	44 4.92	7.88	0.00	0.714	25.50	0.305	10.89	0.409	14.61	57.3		
	MPD.	9 5.03	8.17	57.50	0.00	0.800	28.57	0.315	11.25	0.485	17.32	60.6	0.20		
	M.	29 4.53	7.78	0.00	0.581	20.75	0.221	7.89	0.360	12.86	62.0		
	M.	23 4.53	7.84	0.00	0.790	28.21	0.300	10.71	0.490	17.50	62.0		
	M.	179 4.72	7.83	67.62	0.00	0.699	24.96	0.210	7.50	0.489	17.46	70.0	0.11		
	M.	30 4.58	7.79	0.00	0.597	21.32	0.150	5.36	0.447	15.96	74.9		
	C	M. 151/152	5.20	7.75	51.59	0.00	0.545	19.46	0.307	10.96	0.238	8.50	43.7	0.21	
M.		38 5.32	7.88	37.80	0.00	0.553	19.75	0.245	8.75	0.308	11.00	55.7	0.23		
M.		156 4.48	59.50	0.00	0.785	28.04	0.219	7.83	0.566	20.21	72.1	0.13		
Central Plain															
F	A.	49 6.87	8.21	12.38	0.00	0.126	4.50	0.075	2.68	0.051	1.82	40.5	0.22		
	A.	50 6.31	8.28	14.38	0.15	0.152	5.43	0.069	2.46	0.083	2.97	54.6	0.17		
	A.	66 5.61	8.13	41.88	0.00	0.463	16.54	0.176	6.29	0.287	10.25	62.0	0.15		
	A.	57 6.00	8.17	15.13	0.00	0.255	9.11	0.089	3.18	0.166	5.93	65.1	0.21		
	A.	56 5.93	8.37	13.63	0.00	0.244	8.71	0.075	2.68	0.169	6.03	69.2	0.20		
G	W.	62 6.15	7.91	56.25	0.00	0.739	26.39	0.455	16.25	0.284	10.14	38.4	0.29		
	CPD.	3 6.30	7.95	52.02	0.00	0.562	20.07	0.309	11.04	0.253	9.03	45.0	0.21		
	CA.	24 7.7	7.75	75.75	0.00	0.860	30.71	0.380	13.57	0.480	17.14	55.8	0.18		
	W.	106 4.72	7.80	70.00	0.00	0.716	25.57	0.315	11.25	0.401	14.32	56.0	0.16		
	WOA.	341 6.06	8.04	7.12	0.00	0.220	7.86	0.094	3.36	0.126	4.50	57.3	0.47		
	WOPD.	54 91	8.26	55.12	0.00	0.537	19.18	0.222	7.93	0.315	11.25	58.7	0.14		
	B.	21 5.36	8.15	30.70	0.00	0.549	19.61	0.217	7.75	0.332	11.86	60.5	0.25		
	CA.	14 7.0	7.74	77.90	0.00	0.965	34.46	0.364	13.00	0.601	21.46	62.3	0.16		
	B.	25 4.98	8.12	22.35	0.00	0.398	14.21	0.144	5.14	0.254	9.07	63.8	0.23		
H	WOPD.	3 5.06	8.07	51.62	0.00	0.647	23.11	0.179	6.39	0.468	16.72	72.3	0.12		
	WOA.	320 6.41	7.99	43.10	0.00	0.525	18.77	0.301	10.75	0.224	8.02	42.7	0.25		
	WOA.	339 6.14	7.89	38.00	0.00	0.539	19.25	0.251	8.96	0.288	16.37	53.4	0.23		
	WOA.	332 6.49	8.12	38.00	0.00	0.501	17.89	0.194	6.93	0.307	10.96	61.3	0.18		
Mean value.....												56.2			

* C = clay; F.S. = fine silt.

$\frac{U_3}{U_5}$ and $\frac{U_4}{U_5}$ differ but little from 0.92, 0.96 and 0.99, respectively. The differences between the three values for each ratio are not significant, unless the standard deviations of U_1, U_2, \dots, U_5 are each less than 1 per cent when calculated as percentages of the means. The consistency exhibited by the members of each set of the successive ratio values $\frac{U_1}{U_5}, \frac{U_2}{U_5}, \frac{U_3}{U_5}$, and $\frac{U_4}{U_5}$ together with the fact that measurements of the degree of unsaturation can be reproduced with fair exactness, suggests that they can be used with a reasonable degree of accuracy for the comparative differentiation and characterization of soils, even though they are calculated from data obtained by incomplete extraction. Further, as the values for $\frac{U_1}{U_5}$, for the three soils examined differ no more greatly from one another than those for $\frac{U_2}{U_5}, \frac{U_3}{U_5}$, or $\frac{U_4}{U_5}$, little appears to be gained by continuing the leaching process beyond one liter of filtrate for comparative measurements of this soil factor. In the investigations subsequently described it has been determined on the basis of a 2-liter extraction, for the sake of uniformity with the measurements of saturation capacity and saturation deficit.

The data recorded in tables 3, 4, and 5 demonstrate that the values obtained for the degree of unsaturation, during the routine examination of a large number of soils, cover a wide and satisfactory range. The procedure adopted therefore complies with the third of the conditions regarded as necessary for its use in soil classification.

PART II.—STATE OF UNSATURATION OF THE SOIL IN RELATION TO ITS FIELD BEHAVIOR

DESCRIPTION AND CLASSIFICATION OF THE SOILS EXAMINED

The sugar-cane regions of Trinidad were deemed ideal for the investigation of any relationship which may exist between the state of unsaturation of the soil and its field behavior and lime requirement, first, because of the extreme variations in soil type, and in some cases within the soil type, which occur in a restricted area; and secondly, because one crop is common to them all. Samples, taken to a depth of one foot, representative of the following eight distinct geological types were chosen for examination. Some of these types have been previously described (35).

Soil types of the Naparima District

- A. A brown, raw clay, characterized by the greenish hue of its partially weathered sub-soil. It is for the most part foraminiferal.
- B. A typical, red-weathering, foraminiferal clay, which frequently merges imperceptibly into the previous type.

- C. An old river alluvium, yellow-brown.
- D. A recent river and lagoon alluvium, dark brown.
- E. A black soil, overlying a buff colored calcareous marl.

Soils of the Central Plain

- F. Red-brown detritus soils, bordering on the foothills of the Northern Range, and derived from the Pre-Cretaceous quartzose schists of which the range is mainly formed.
- G. Fawn colored, one-time swamp and lagoon alluvial soils, which merge into present-day swamps.
- H. Dark brown, recent river alluvial soils.

All the foregoing types are essentially alumino-siliceous in character and are very deficient in organic matter (35). Some of them have been more fully described by Hardy (15).

System of classification

The samples obtained from the eight soil types are classified into three groups, according to their field behavior:

Group I. Soils possessing an inherent good tilth.

Group II. Soils with neither markedly good nor bad properties, but which are beginning to exhibit the characteristics of physical deterioration, and are at present in the transition stage from the first to the third groups.

Group III. Soils in a badly impaired condition, to which, at the best, a temporary good tilth only can be given.

The climatic conditions prevailing in Trinidad emphasize the differences in behavior of the soils of groups I and III and render them readily distinguishable. Briefly it may be stated that the latter exhibit marked fluctuations in behavior from the dry to the wet season, appear to be poorly aerated, and are in obvious need of flocculation. The variation in behavior of the former from season to season is much less pronounced, and they possess, throughout the year, as good a tilth as climatic conditions and their content of organic matter appear to allow. A further and almost invariable distinction is that the soils of the third group support sugar canes periodically subject to severe injury from an insect pest—*Monecphora (Tomaspis) saccharina* Dist. (the frog-hopper)—from which those grown on the soils of the first group are almost completely free. Recently in years of serious insect infestations, canes grown on the second group have been subject to slight damage in this manner.

In addition to the measurements necessary for the determination of the state of unsaturation of the soils of the three groups, the following determinations were made:

pH value was measured on duplicate representative samples of each soil by means of the quinhydrone electrode. A soil-water ratio of 1 to 2 was used.

The soil content of *clay and very fine silt* was determined previous to the publication of the

recommendations of the Agricultural Educational Association in 1928. It was measured according to the procedure adopted by this body in 1925. The necessary corrections for temperature were made.

Calcium carbonate was estimated by means of Collin's calcimeter.

The data obtained for 50 samples, selected from those examined as representative of the good, transitional, and poor soils of the eight types described in the foregoing, are summarized in tables 3, 4, and 5, respectively. The soils of each group are arranged in order of increasing degree of unsaturation. Type B, which is fairly uniform in saturation capacity, and type G, which is very variable in this respect, because of their diverse origin, are more fully exemplified than the other types.

TABLE 6

Distribution of saturation capacities (T), contents of exchangeable calcium (S) and saturation deficits (T-S) of soils of Groups I, II, and III

CaO PER 100 GM AIR-DRY SOIL	SOILS IN GOOD CONDITION GROUP I			TRANSITION SOILS GROUP II			SOILS IN POOR CONDITION GROUP III		
	T	S	T-S	T	S	T-S	T	S	T-S
0.0-0.1	0	0	3	0	0	1	0	5	2
0.1-0.2	0	0	7	0	0	1	2	5	3
0.2-0.3	1	3	1	0	1	3	3	7	9
0.3-0.4	2	1	0	1	1	1	1	11	8
0.4-0.5	1	3	1	0	3	0	1	4	8
0.5-0.6	3	1	0	1	0	0	11	0	1
0.6-0.7	0	0	0	3	1	0	3	0	1
0.7-0.8	1	0	0	0	0	0	6	0	0
0.8-0.9	0	1	0	0	0	0	4	0	0
0.9-1.0	1	2	0	1	0	0	1	0	0
>1.0	3	1	0	0	0	0	0	0	0
Total...	12			6			32		

SATURATION CAPACITY, EXCHANGEABLE CALCIUM, AND SATURATION DEFICIT

The distribution of the saturation capacities, the contents of exchangeable calcium, and the saturation deficits of the soils of groups I, II, and III are recorded in table 6.

Saturation capacity

The saturation capacities of the soils of group I are fairly evenly scattered between the values 0.25 and 1.7 gm. CaO per 100 gm. of air-dry soil; those for the soils of group II range from 0.3 to 1.0 per cent; whereas the majority of the values for the soils of group III lie between 0.5 and 0.9 per cent. No regular relationship appears to exist between field behavior of the soil and the magnitude of the saturation capacity, provided it is greater than 0.25 per cent

CaO. High saturation capacities, such as are characteristic of types A and B and some of the soils of type G, are not necessarily correlated with poor physical condition, for the soils of type E, which possess even higher saturation capacities, retain their tilth throughout the year more markedly than do any of the soils of the other seven types. Between poor condition and low ion capacity there may be some connection. The minimum saturation capacity found for the good and transitional soils is 0.25 per cent CaO. The behavior of the soils which possess values below this amount suggests that their condition is in part due to the lack of sufficient colloidal material successfully to withstand the climatic conditions which prevail.

Exchangeable calcium

The contents of exchangeable calcium of the soils examined range from 0.075 to 1.298 gm. CaO per 100 gm. of air-dry soil. This range is greater than that found by Williams (37) for Welsh soils (0.07 to 0.28 per cent CaO), by Smith (33) and Ogg and Dow (27) for Scottish soils (0.04 to 0.55 per cent CaO), by Kelley and Brown (23) for American soils (0.12 to 0.92 per cent CaO), and by Hissink (16) for Dutch soils (0.21 to 1.12 per cent CaO).

The content of the soils in good condition may rise above 1.0 per cent CaO, whereas the maximum values recorded for the transition and poor soils examined are 0.7 and 0.5 per cent, respectively. Apart from these limiting cases there is little evidence of a relationship between the absolute amount of exchangeable calcium present and field behavior. This appears to be mainly due to the variation which exists in the saturation capacities of the soils examined, for an increase in the capacity for ion exchange of the good soils is invariably accompanied by a concurrent increase in their contents of exchangeable calcium.

Saturation deficit

To some extent account is taken, in the determination of saturation deficit, both of the saturation capacity of the soil and of its content of exchangeable calcium. A closer relationship therefore may be expected between it and soil behavior than exists in the case of either of the factors from which it is derived. Certain investigators have used it as a measure of the lime requirement of the soil. Those methods for determining lime requirement which involve the estimation of the acidity developed by the soil in the presence of neutral salt solutions are based upon qualified measurements of its value. The degree to which the magnitude of the saturation deficit is linked with the field behavior of the soils examined is therefore worthy of detailed consideration.

The saturation deficits registered in tables 3, 4, and 5 vary from 0.04 to 0.60 gm. CaO per 100 gm. of air-dry soil; those for the soils of group I lie between 0.04 and 0.42 per cent CaO; whereas the values for the soils of group III cover the whole of the recorded range. In the great majority of cases, however, the

saturation deficits of the soils of the former group are less than 0.20 per cent CaO, whereas those for the soils of the latter group are in general larger than this. The deficits of the transition soils are ranged about this value, varying from 0.10 to 0.31 per cent CaO. A loose relationship, therefore, appears to exist between the saturation deficits of the soils as a whole and their field behavior, but the overlap which occurs in the values for individual soils of the different groups renders the connection too ill defined to warrant a formulated generalization. The lack of precise definition which obtains is well illustrated by the following specific examples. Soil M 42 of type E, group I, has a saturation deficit (0.42 per cent CaO) greater than the values for all the soils of group II and for most of the soils of group III. Similarly, the deficits of soils A 49 and A 50, of type F, group III, (0.05 and 0.08 per cent CaO, respectively) are smaller than those for the vast majority of the soils of groups I and II. These are extreme cases, but many examples similar in kind, though of lesser degree, appear in the tables.

The data obtained nevertheless demonstrate that in special cases, such as those for soils confined to a single type which is of fairly uniform saturation capacity throughout, a close relationship may exist between saturation deficit and field behavior. For example, the minimum deficit found for the poor soils of type C is 0.24 per cent CaO, the transition soil has a value of 0.18, and the good soils of this type possess deficits which range from 0.12 to 0.15 per cent CaO. The soils of the different groups of types B and H exhibit a similar gradation in their limiting values. When, however, the saturation capacity of the type is very variable, the relationship becomes less well defined. In the case of type G, the poor soils have a minimum deficit of 0.13, the good soils a maximum deficit of 0.14, and the transition soil cited possesses the value 0.23 per cent CaO.

It is evident from these examples that, as in the case of exchangeable calcium, the relationship between the magnitude of the saturation deficit and the field behavior of the soil is obscured by the variation which occurs in the capacity of the soil for ion exchange. If the saturation capacity is very high, a deficit of 0.4 per cent CaO is not incompatible with inherent good tilth. If, on the other hand, it is very low, as small a deficit as 0.05 per cent CaO may be correlated with poor condition. It follows that the saturation deficit cannot, in general, be regarded as a reliable index of soil condition.

DEGREE OF UNSATURATION

The degree of unsaturation of the soils examined varies from 12 to 88 per cent (tables 1, 3, 4, and 5). This range is wider than that found by Hissink (17) for Dutch soils (44 to 74 per cent), by Joffe and McLean (22) for American soils (12 to 63 per cent) and by Osugi and Sano (28) for Japanese soils (17 to 77 per cent).

The extent to which the Trinidad soils classified in group III have become

unsaturated appears independent of internal factors such as the magnitude of their saturation capacity and the nature of the type to which they belong. No evidence exists that the light soils of type G, for example, are in general more highly unsaturated than its heavy soils, or that the soils of type F are less saturated than those of type B.

Although no close connection exists in general between the field behavior of the soil and its absolute content of exchangeable hydrogen or calcium, the data in tables 3, 4, and 5 show it to be intimately related to their ratio value. The soils in good condition, those with a somewhat impaired tilth, and the poor soils each lie within a fairly well-defined zone of unsaturation. The degree of unsaturation of the soils in good condition ranges from 12 to 28 per cent, with a mean value of 21 per cent. The limits of unsaturation of the transition soils are 30 and 35 per cent, with a mean value of 32 per cent. The soils in poor condition are more than 38 per cent unsaturated.

The delimiting values of the three zones appear to be independent of the following factors:

The nature of the colloidal material of the soils examined, as determined by the type to which they belong. Each range of values holds for the soils of all eight types, in spite of their diverse origin.

The degree of the colloidity of the soils, whether measured by their saturation capacity or their content of clay and fine silt. The soils of the very variable type G are as precisely differentiated according to their condition as the soils of the more uniform types.

Any variation which occurs in the content of exchangeable ions, other than hydrogen and calcium, of the soils examined.

The agricultural history of these soils, which in some cases have grown cane for generations, whereas others have until recently supported cacao.

The degree of unsaturation with calcium, i.e., the ratio of exchangeable hydrogen to calcium, therefore appears to be a master factor controlling the field behavior of these soils.

It is known that humus, if present in large quantities, may dominate the properties of the soil and mask the effect of the exchangeable ions, but that, if it occurs to a small extent, the nature of its influence is largely dependent upon their relative proportions. The amount of organic colloidal material found in the soils of the cane-growing regions is relatively small compared with the high degree of inorganic colloidity possessed by many of these soils (35). This may aid in emphasizing the measure of control over the soils examined which the degree of unsaturation exhibits, and may afford an explanation of the comparative precision with which the zones of unsaturation are defined. It is noteworthy in this respect that Gehring, Peggau, and Wehrmann (12) found that lime requirements, based on their method of determining the degree of saturation, are applicable to mineral but not humic soils.

It has been shown in a previous paper (35) that the soils of Trinidad in poor condition are distinguished from those in good condition by the ratio of the amount of exchangeable calcium present in them to their content of clay and

very fine silt (C. + F.S. II). The ratios for the former soils were found to range from 0.11 to 0.25, and those for the latter from 0.28 to 0.63. The ratios for the extended series of soils, recorded in column 12 of tables 3 and 5, confirm these findings. They vary from 0.11 to 0.29 for the soils of group III and from 0.28 to 0.68 for those of group I. The contrasted soils are not so clearly differentiated by this means, however, as by their degree of unsaturation, for the ratios for the transition soils, which vary from 0.28 to 0.38, lie within the range for the good soils. Further, the poor soil WOA 341 of type G, originally found to be an exception in that it has a ratio value of 0.47, possesses a degree of unsaturation of 57 per cent, a value well within the zone corresponding to the soils of group III.

Hissink (19) found the ratio of exchangeable calcium to clay and very fine silt for Dutch soils to be highly correlated with their degree of saturation.

TABLE 7
Degree of unsaturation of new, medium, and old Dutch soils
[Recalculated from the data of Hissink (17)]

Soil number..	AGE OF SOIL									
	New			Med- ium	Old and very old					
	B1681	B1459	B1680	B1679	B790	B1482	B1483	B1484	B1458	
100 (T-S)/T										
Hissink's value.....	43.6	47.2	45.5	48.0	53.2	61.2	59.2	55.3	74.2	
Recalculated value.....	49.2	52.8	51.1	53.6	58.7	66.4	64.5	60.8	78.2	
pH value.....	7.61	7.57	7.70	7.67	7.03	5.89	5.96	6.25	7.49	
Humus (per cent.).....	0.0	0.7	0.0	1.5	3.2	3.4	3.1	2.1	0.0	
CaCO ₃ (per cent.).....	10.6	9.3	3.2	0.7	0.0	0.0	0.0	0.0	0.0	

The correlation of $\frac{S}{(C. + F. S. II)}$ with $\frac{100(T - S)}{T}$ for the 41 Trinidad soils for which both sets of values have been obtained is -0.726 , with $P = <0.01$.

COMPARISON WITH RESULTS OF HISSINK

Hissink (17) included the soil content of exchangeable sodium, potassium, and magnesium in his measurement of saturation capacity. For the purpose of comparison his data have been recalculated on the assumption that calcium and hydrogen alone are present. The recalculated degrees of unsaturation are each greater than the original values by from 4 to 6 per cent.

The zones of unsaturation of the new and old soils of Holland are limited by the values 49 and 53 per cent, and 58 and 78 per cent, respectively. Although the new soils contain calcium carbonate, their degree of unsaturation lies well within the zone which characterizes the poor soils of the cane-growing regions of Trinidad. It is clear, therefore, that the method of Hissink for determining

the soil content of exchangeable hydrogen gives much higher values than the modified method of Page and Williams.

It is doubtful whether the divergence can be explained either by the difference in the humus contents of the two series of soils—for the contents of the Dutch soils, which were determined by loss in weight on ignition, are the smaller—or by the difference in the length of time allotted to replacement in the two processes. In the modified method of Page and Williams the leaching process alone, in which the soil is repeatedly brought into contact with fresh sodium chloride solution, normally takes at least 3 days.

To investigate the reason for the discrepancy, the saturation deficits of the three soils of types F, G, and B, samples of which had previously been leached to five liters, were determined by the method of Hissink. The pro-

TABLE 8

Values for $(T-S)$, T , and $\frac{100(T-S)}{T}$ as determined by the method of Hissink and by the modified method of Page and Williams (2- and 5-liter extractions)

SOIL TYPE	METHOD OF HISSINK			MODIFIED METHOD OF PAGE AND WILLIAMS									RATIO OF VALUES FOR (T-S)	
	1	2	3	4	5	6	7	8	9	(1)/(4)	(1)/(7)			
	(T-S)	T	$\frac{100(T-S)}{T}$	2 liter extraction			5 liter extraction							
				(T-S)	T	$\frac{100(T-S)}{T}$	(T-S)	T	$\frac{100(T-S)}{T}$					
mgm. E. per cent	mgm. E. per cent	per cent	mgm. E. per cent	mgm. E. per cent	per cent	mgm. E. per cent	mgm. E. per cent	per cent						
F	10.57	13.03	81.1	3.43	5.89	58.2	4.25	6.71	63.3	3.1	2.5			
G	47.23	55.16	85.6	17.57	25.50	68.9	23.00	30.93	74.4	2.7	2.1			
B	49.29	60.54	81.4	19.25	30.50	63.1	26.39	37.64	70.1	2.6	1.9			
Mean.....										2.8	2.2			

cedure followed was identical with that described by him. In each case the amount of soil taken was such that it contained approximately 2 gm. of the clay fraction. The soils were placed in a series of test tubes and increasing quantities (5, 10, 15 45 cc.) of 0.1 N Ba(OH)₂ added. Each tube was filled with water to 50 cc., shaken occasionally for three days, and then allowed to settle overnight. A portion of the clear supernatant liquid was pipetted off and titrated with standard hydrochloric acid, phenolphthalein being used as the indicator. From the data obtained the saturation deficits of the three soils were calculated. Their values, together with those obtained by their use for the saturation capacity and degree of unsaturation, are compared in table 8 with the corresponding values given by the modified method of Page and Williams on the basis of 2- and 5-liter extractions.

The values obtained by the method of Hissink for the saturation deficits of

the three soils examined are approximately three times those given by a 2-liter extraction with normal sodium chloride solution, and twice those given by a 5-liter extraction. These results are similar in nature to those of von 'Sigmond (32), who considers that by the method of Hissink an overestimate of the value for $(T - S)$ is obtained. Gericke (13) has shown that the procedure of Hissink gives higher values for saturation capacity than that of Gehring (11) and of Bobko and Askinasi (2).

It will be recollected that in the soil of type F, which is of low saturation capacity, replacement of the ionizable hydrogen by the leaching process appears practically complete when five liters of filtrate are obtained. There is no indication that the value for saturation capacity would increase appreciably in magnitude if leaching were continued indefinitely. In the case of this particular soil, therefore, there is strong evidence that reactions other than those of pure exchange occur in the procedure adopted by Hissink. With the heavier soils of types G and B the evidence is less definite, their saturation capacity values show no marked signs of approaching a maximum value at the 5-liter stage of extraction (table 2). Assuming, however, that further leaching would continue to replace 1.57 and 2.14 mgm. E. of hydrogen per liter (the mean of the amounts removed by the fourth and fifth liters from the soils of types G and B, respectively), a total of 16 and 20 liters of filtrate would be necessary to attain the Hissink values.

pH VALUE

The pH values of the soils examined lie between 4.48 and 7.87. The minimum value recorded for the good soils is pH 5.69, and the maximum value for the poor soils, 6.87. The transition soils possess values which range from pH 5.31 to 6.89. The transition and poor soils are, in general, much more acidic in reaction than the medium aged and old soils, respectively, of Holland (table 7). Hissink (17) found that Dutch soils are frequently alkaline in reaction even though their degree of unsaturation is relatively high.

It is to be expected that the ratio of ionizable hydrogen to ionizable calcium present in the soil will exert some measure of control over its hydrogen-ion concentration when in equilibrium with water. The data accumulated uphold this view, in that there is a general tendency for the pH values of the soils of each type to decrease with an increasing degree of unsaturation. The frequent and striking irregularities which occur, however, both for soils within a single type which exhibit a gradation in unsaturation, and for soils of different types with approximately equal degrees of unsaturation, indicate that other factors of primary importance contribute to the determination of pH value. In consequence, the good, transition, and poor soils are not well defined by their hydrogen-ion concentration. The influence of the degree of unsaturation is nevertheless to some extent predominant, for none of the soils in good condition possess pH values which lie at the very acid end of the scale (pH 4.0 to 5.5), and although an appreciable proportion of the poor soils have values

above pH 6.0, none are actually alkaline in reaction. Further, none of the transition soils possess the extreme values pH 4.0 to 5.0 and pH 7.0 to 8.0.

The content of organic matter of the soil is known to be one of the more important of the disturbing factors which affect pH value. Its action has previously been noted by Hissink (17) and other workers, who attribute to the humus present the abnormally high acidity of soils possessing a relatively low degree of unsaturation with calcium. The data obtained for the soils of the cane-growing regions of Trinidad lead to a similar conclusion. The pH values of two series of soils, selected from those examined because the saturation capacity and degree of unsaturation of the soils of each set are approximately constant, are given in table 9. In both series a marked increase in hydrogen-ion concentration occurs as the proportion of organic matter increases. The

TABLE 9
Effect of organic matter content on pH value

SOIL NUMBER	ORGANIC MATTER	SATURATION CAPACITY (CaO PER CENT)	DEGREE OF UNSATURATION	pH VALUE
	<i>per cent</i>		<i>per cent</i>	
<i>Series I</i>				
WOA. 320	1.25	0.525	42.7	6.41
CPD. 3	2.85	0.562	45.0	6.30
M. 27	4.89	0.579	44.7	5.79
M. 151/152	5.32	0.545	43.7	5.20
<i>Series II</i>				
WOA. 339	1.00	0.539	53.4	6.14
M. 38	3.46	0.553	55.7	5.32
WOPD. 5	4.80	0.537	58.7	4.91

pH value of the soil, therefore, cannot be regarded as a reliable index for soil classification on the basis of field behavior.

PART III.—DETERMINATION OF LIME REQUIREMENT ON THE BASIS OF DEGREE OF UNSATURATION

FACTORS CONTRIBUTING TO THE NEED OF THE SOIL FOR LIME

The ultimate aim of liming is to render the soil more suited to the development in the crop of some particular character or potentiality, such as an increase in yield, an improvement in quality, or a greater resistance to injury from disease or insect pests. This may be attained in one or more of the following ways: by improving the physical structure of the soil; by reducing its degree of acidity to a given value, and incidentally, rendering unexchangeable the ionizable iron and aluminium it contains; or merely by increasing to

a given amount the calcium available to the plant. It is improbable that all crops, and possibly even the same crop under different climatic conditions, will react equally well to any given soil treatment. Further, it is unlikely that each of the three possible courses of action will require an equal amount of lime. It is necessary, therefore, to determine which is most suited to the crop under consideration, under the particular set of conditions which prevail, although the conclusions reached may be of wider application.

Sugar canes in Trinidad, under normal circumstances grow almost equally well on the majority of soils examined, provided that due care is taken in selecting the variety most suited to local conditions, and that the soil is suitably tilled and drained and contains at least 0.2 per cent exchangeable calcium (35). Sugar canes as a class are therefore very tolerant of considerable variation in the physical characteristics of the soil, in its state of unsaturation and degree of acidity. As in the case of other crops (1, 4, 18, 20) it is under adverse circumstances, such as extended periods of meager rainfall or serious infestations of insect pests, that among them marked differences in behavior appear. Then the canes grown on the soils classified in group III are liable to a severe setback, and those on the soils of group II to slight injury, whereas the canes on the soils of group I as a rule remain practically unscathed. Field observations and the data accumulated in the laboratory indicate that it is by their field behavior, rather than by their degree of acidity or their content of exchangeable calcium, that the soils of the three groups are essentially differentiated. In this particular case, therefore, it is primarily to amend their physical condition that the soils of groups II and III appear to need lime.

Under circumstances such as these the manner in which the good soils are differentiated from the others by their degree of unsaturation becomes of practical importance, for once the content of exchangeable calcium and the saturation capacity of a soil are known, it is a matter of simple calculation to determine the lime required to bring its degree of unsaturation within the zone which characterizes the good soils.

FACTORS DETERMINING THE DEGREE OF UNSATURATION OF THE SOILS IN GOOD CONDITION

Equilibrium position of the system soil-calcium carbonate

In addition to the dissimilarity in their degree of unsaturation, the soils in good and poor condition differ outstandingly in that the good soils have been in contact with calcium carbonate for a long period of years. The soils of types D and E of group I are naturally calcareous, whereas those of types C, G, and H, which are classified in this group, have been liberally limed in the past. All these soils, excluding M 40, the store of calcium carbonate of which has but very recently become exhausted, still possess a reserve of limestone. The soils of type E, and those of type D in good condition, with this one possible exception, are undoubtedly in equilibrium with this substance, as in all

probability are the other soils of group I. Such soils were believed by Ramann (31) and Gedroiz (10) to be saturated with calcium, and they are still so considered by some workers (24). Nevertheless, as the data in table 3 demonstrate, they contain exchangeable hydrogen.

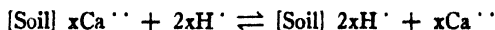
The presence of ionizable hydrogen under these conditions is to be expected on theoretical grounds. The compounds formed by the action of calcium carbonate on the acidoid material of the soil are salts of a relatively strong positive ion and a very weak colloidal negative ion, and as such they are prone to hydrolysis. Should the reaction go to completion the normal salt so formed would be unstable, and interaction between it and the hydrogen ions of the soil water would occur. The latter process would result in the formation of unionized (but ionizable) soil acid, and this would proceed until equilibrium conditions were attained.

It appears from these considerations that the saturation deficits of the good soils are measures of the extent to which hydrolysis can occur in them when they are in equilibrium with calcium carbonate, although, as the estimation procedure used does not completely displace the ionizable hydrogen present, the values obtained somewhat underestimate its scope. Similarly their degree of unsaturation is a measure of the proportion of acidoid material unneutralized under these conditions.

These conclusions clarify the meaning of the data obtained. They demonstrate that the amount of lime required completely to neutralize the acidoid material of the soil is in excess of that with which it can normally combine. They show that the degree of unsaturation of the good soils is a measure of the amount of calcium with which they can stably unite, rather than a measure of the point at which the ratio of exchangeable calcium to exchangeable hydrogen is such that the calcium ion exerts a predominating influence over the properties of the soil, although in the good soils such a state of affairs may exist. Further, they indicate that estimations of lime requirements on the basis of the degree of unsaturation of the good soils are essentially determinations of the amounts of lime necessary to bring the soil into equilibrium with calcium carbonate.

Variation in equilibrium position with the soil

The limiting values found for the degree of unsaturation of the good soils (12 and 28 per cent) suggest that a considerable degree of variation with the soil occurs in the proportion of hydrolyzed material present under equilibrium conditions. The data in table 1 demonstrate that the difference between the maximum and minimum values obtained lie well outside the range of experimental error.



(88–72 per cent)

(12–28 per cent)

The deviations from a constant value may be attributed to one or more of the following factors:

First, the presence in the soil of calcium carbonate, which will to some extent pass into solution during the determination of exchangeable calcium, and thus affect the value for the degree of unsaturation.

In selecting samples of the good soils for examination, those with the smallest contents of calcium carbonate were chosen, to reduce this error to a minimum. In consequence, with few exceptions, the amounts present in the soils classified in table 3 are too small appreciably to influence the values obtained.

Secondly, variation in the strength of the acidoid material, both inorganic and organic, with soil type.

Evidence has previously been obtained that under comparable conditions the acidoid complex varies in the extent to which it ionizes in soils of different types (34). A similar variation is shown by the values for the degree of unsaturation of the types of group I. The mean degrees of unsaturation of the soils, which appear to be in equilibrium with calcium carbonate, of types C, E, G, and H, are 21, 22, 26, and 15 per cent, respectively. The difference between the mean values for types G and H is 11 per cent, its standard error being 4.3 per cent. This difference is therefore significant. A fuller exploration of the cause and range of variation within each type is needed, however, before the meaning of such a difference can be accurately assessed.

Thirdly, lack of consistency in the soil content of humified organic matter.

This may be an important factor contributing to the differences found, but insufficient data have been accumulated for an estimation of its effect.

NET AMOUNT OF LIME REQUIRED FOR ATTAINMENT OF EQUILIBRIUM WITH CALCIUM CARBONATE

For reference, table 10 gives the net amounts of lime required to bring into equilibrium with calcium carbonate soils with saturation capacities that range from 0.1 to 1.0 per cent CaO, and with degrees of unsaturation that vary from 20 to 100 per cent. The calculations are based on three assumptions: first, that the degree of unsaturation corresponding to the equilibrium position is 20 per cent, a value which closely approximates to the mean for the soils of group I; secondly, that no loss of lime occurs through soil wash or leaching in the period during which it is reacting with the soil; and thirdly, that an acre-foot of soil weighs 4,000,000 pounds. The examples recorded are classified in accordance with the field behavior of the soils.

When account is taken of the saturation capacity and state of unsaturation of the examples recorded in table 10, the amounts of lime required for the attainment of a state of equilibrium do not appear excessive in magnitude. In rather more than one-half of the cases cited they are less than 5 tons an acre. They exceed 10 tons only when the saturation capacity of the soil reaches a value of 0.4 per cent CaO, and then only when the degree of unsaturation is relatively high. Quantities larger than 20 tons are needed only if a saturation capacity of 0.8 per cent, or more, coexists with a degree of unsaturation greater than 80 per cent. Soils more than 70 per cent unsaturated are uncommon in the cane-growing areas of Trinidad, in spite of the heavy tropical

rainfall and the exhaustive methods of cultivation which have been practiced for generations (36). They are probably of even less frequent occurrence in temperate zones. According to the data in table 10, therefore, in the great majority of cases the amount of lime needed is restricted to a maximum of 16 tons of calcium carbonate to the acre, unless the saturation capacity of the soil exceeds 1 per cent CaO. Of the 50 samples for which data are given in tables 3, 4, and 5, 29 possess requirements less than 5 tons an acre, 14 from 5 to 10 tons, and 7 from 10 to 13 tons.

This method for determining net lime requirement is independent of the presence of calcium carbonate in soils not in equilibrium with calcium carbonate. In such cases the value found for the degree of unsaturation will lie within the zone of unsaturation of the soils of group I only if the amounts

TABLE 10
Net amounts of lime, in tons CaCO_3 per acre, required to bring the soil into equilibrium with calcium carbonate

SATURATION CAPACITY (PER CENT CaO)	GOOD SOILS	TRANSITION SOILS		SOILS IN POOR CONDITION						
	Degree of unsaturation (per cent)									
	20	30	35	40	50	60	70	80	90	100
0.10	0	0.32	0.48	0.64	0.96	1.23	1.59	1.91	2.23	2.55
0.20	0	0.64	0.96	1.28	1.91	2.55	3.19	3.83	4.46	5.10
0.30	0	0.96	1.44	1.91	2.87	3.83	4.78	5.74	6.69	7.65
0.40	0	1.28	1.92	2.55	3.83	5.10	6.38	7.65	8.93	10.20
0.50	0	1.59	2.39	3.19	4.78	6.38	7.97	9.56	11.16	12.75
0.60	0	1.91	2.87	3.83	5.74	7.65	9.56	11.48	13.39	15.30
0.70	0	2.23	3.35	4.46	6.69	8.93	11.16	13.39	16.62	17.85
0.80	0	2.55	3.83	5.10	7.65	10.20	12.75	15.30	17.85	20.40
0.90	0	2.87	4.31	5.74	8.61	11.48	14.35	17.22	20.08	22.95
1.00	0	3.19	4.78	6.38	9.56	12.75	15.94	19.13	22.32	25.50

present are in excess of those necessary for the attainment of a state of equilibrium. If present in quantities smaller than this, the additional quantities required can be calculated in the normal manner from the apparent degree of unsaturation found.

NEED FOR A RESERVE OF CALCIUM CARBONATE IN THE SOIL

Repression of ionization

The fact that the degree of unsaturation of the soils in good condition appears to be a measure of the equilibrium position of the system soil-calcium carbonate, rather than an indication of the point at which the soil content of exchangeable calcium is large enough to impress the characteristics of a calcium

clay upon the soil, renders it doubtful whether differences in the degree of unsaturation alone, always afford a complete explanation of the dissimilarity in the behavior of the soils examined, and suggests that the calcium carbonate of itself may, under certain conditions, play an important part. The relatively small differences in the degree of unsaturation of the soils of groups I and II, in comparison with the contrast displayed by their properties, tend to confirm this possibility. It seems likely, in the case of the soils of group I, that the calcium ions, to which the calcium carbonate gives rise, suppress in large measure the ionization of the calcium salts of the acidoid material of the soil, thereby acting as a coagulating agent. In the soils of group II (and those of group III) ionization can occur unhindered, and this may contribute materially to the deterioration evident in their physical structure. The action of the ions derived from the calcium carbonate would thus be akin to the repression of the ionization of an ordinary electrolyte, by the mass influence of an added ion common to both electrolytes.

Maintenance of the degree of unsaturation at equilibrium value

It has been pointed out by Hissink (17) that the soils of Holland of medium age differ but little in their degree of unsaturation from those newly endyked (table 7). Both series of soils contain calcium carbonate, but the amounts present in those of medium age are much the smaller. This substance is absent from the old and very old soils, which tend to be much more highly unsaturated than the soils of the other groups. Hissink concludes that calcium is lost through leaching originally at the expense of calcium carbonate present, and that when it is exhausted, losses of exchangeable calcium from the clay material begin. The data recorded in tables 3, 4, and 5 lead to a similar conclusion. They indicate that the degree of unsaturation of the soils of group I is maintained by their store of calcium carbonate at a value which approximates 20 per cent, and that it is not until the store is consumed that the degree of unsaturation commences appreciably to increase.

It is probable that the calcium removed from the soil by leaching is initially present in solution either as unionized calcium bicarbonate or in the form of ions. The ions are probably derived chiefly from the dissolved calcium salt of the relatively strong bicarbonate ion, rather than from the very slightly ionized salts of the weakly acidic clay complex. If the state of equilibrium in the soil solution is displaced through loss under these circumstances, it would be restored almost entirely by further ionization of the calcium bicarbonate, which in turn would be replaced by solution of the calcium carbonate present,

It appears advisable, therefore, both from the point of view of repression of ionization and the maintenance of the soil content of exchangeable calcium, to make provision in estimates of lime requirement for amounts of calcium carbonate in excess of those necessary for the creation of equilibrium conditions. The excess included for the purpose of forming a reserve must necessarily be

TABLE 11

Comparison of net lime requirements as determined by (a) pH value method of Hardy and Lewis and (b) method of degree of unsaturation

SOIL TYPE	SOIL NUMBER	TONS CaCO ₃ REQUIRED TO BRING		RATIO B/A
		(A) pH to 7.0*	(B) Degree of unsaturation to 20 per cent	
Group I. Soils in good condition				
C	M. 64	1.08	0.00	0
	M. 101	0.54	0.41	0.76
D	M. 181	1.08	0.00	0
E	MPD. 7	0.93	0.03	0.03
G	CA. 15	1.31	0.61	0.47
	CPD. 1	0.41	0.86	2.10
	CA. 16	0.67	1.05	1.57
H	O. 59	2.74	0.00	0
	A. 10	0.80	0.00	0
Mean value.....				0.55
Group II. Transition soils				
C	M. 71	2.55	2.30	0.90
G	W. 48	1.40	2.74	1.96
H	O. 122	2.04	1.05	0.51
Mean value.....				1.12
Group III. Soils in poor condition				
A	M. 50	4.40	7.37	1.68
B	MPD. 9	6.63	10.36	1.56
	W. 62	2.46	4.34	1.76
G	CPD. 3	2.74	4.50	1.64
	CA. 2	10.00	9.82	0.98
	W. 106	9.57	8.23	0.86
	WOA. 341	1.66	2.62	1.58
	WOPD. 5	8.71	6.63	0.76
	CA. 1	11.16	13.01	1.17
	WOPD. 3	8.61	10.81	1.26
	WOA. 320	3.25	3.79	1.17
H	WOA. 339	3.51	5.74	1.64
	WOA. 332	4.82	6.60	1.37
Mean value.....				1.34

* The lime requirements in this column were calculated from data provided by Prof. F. Hardy.

greater than losses due to the action of rain water over the period during which the soil content of exchangeable calcium is being increased to its maximum value. In Great Britain these losses are estimated to amount to half a ton an acre (26). In the humid tropics they are undoubtedly larger, but their actual magnitude is as yet unknown.

COMPARISON WITH RESULTS OBTAINED BY THE pH VALUE METHOD OF HARDY AND LEWIS

Hardy and Lewis (14) have devised a method for determining the amount of lime required to reduce the acidity of soils to a pH value of 7.0. According to their procedure 10 gm. of soil is mixed with 40 cc. of neutral 0.2 N CaCl_2 solution and titrated with 0.03 N $\text{Ca}(\text{OH})_2$, which is added in successive portions of 5 cc. at 3-minute intervals with shaking. The pH value is determined after each addition by means of the quinhydrone electrode, and the titration is continued until an alkaline reaction is obtained. The results are plotted in the form of a graph, from which the amount of calcium hydroxide, and hence calcium carbonate, required to give a pH value of 7.0 is read. For the purpose of comparison, table 11 records the net lime requirements of a selected series of soils, as determined by the method of unsaturation and by the alternative method of pH value of Hardy and Lewis.

The data in this table indicate that:

The net lime requirements of the soils of group I are larger when determined by the method of Hardy and Lewis than by the method of unsaturation. This may be explained on the grounds that although these soils are in equilibrium with calcium carbonate, they still contain readily exchangeable hydrogen, which is in part replaced by the treatment with calcium chloride and calcium hydroxide.

The net lime requirements of the soils of group III, on the other hand, as a rule are considerably smaller when determined according to the pH value method than according to the method of unsaturation. This suggests that the amount of hydrogen replaced by the former method from soils in a condition of relatively high unsaturation is in general less than that required to reduce their degree of unsaturation to 20 per cent.

The lime requirements as given by the two methods for the 16 soils of groups II and III are highly correlated, r being equal to $+0.911$, with P less than 0.01.

SUMMARY

There is considerable evidence that hydrogen and calcium together account for the great majority of the exchangeable ions present in the soils of humid climates. Upon the relative proportions in which these ions occur, and possibly also on their absolute amounts, the properties of such soils may be expected to depend. An investigation has therefore been made of the extent to which the calcium-hydrogen status of a series of soils is related to their field behavior, and upon the conclusions reached a method for determining lime requirement has been based.

1. Exchangeable calcium and hydrogen together (saturation capacity) were determined by leaching to two liters according to the modified method of Page and Williams. Exchangeable calcium was measured by the method of Hissink, and exchangeable hydrogen (saturation deficit) determined by difference. From these data the degree of unsaturation was calculated.

A critical study of the procedure used has shown that:

The mean values obtained for saturation capacity, exchangeable calcium, saturation deficit, and degree of unsaturation, from duplicate estimations on a single subsample, give a sufficiently close approximation to those obtained from the examination of a number of subsamples for most purposes of soil differentiation and characterization.

Only partial replacement results from leaching to two liters. Measurements of the degree of unsaturation based on a 2-liter (or possibly even a 1-liter) extraction may nevertheless be used with a fair degree of confidence in comparative studies of soils, for, (a) constant results are obtained on repetition for the amounts of calcium and hydrogen removed, (b) the ratios of the values for degree of unsaturation, determined on the basis of 1-, 2-, 3-, and 4-liter extractions, to those given by a 5-liter extraction, closely approximate constants, apparently independent of the type and saturation capacity of the soil, and (c) exchange of hydrogen occurs to such an extent that the ratios of replaced hydrogen to calcium in good and poor soils are sufficiently different adequately to reflect the dissimilarity in their properties.

In the case of saturation capacity and saturation deficit the ratios of the values given by 2-liter and 5-liter extractions decrease significantly with increasing saturation capacity. The measurements obtained for saturation capacity and saturation deficit may therefore not be strictly comparable.

II. The samples taken from the eight soil types examined were classified into three groups:

Group I. Soils possessing an inherent good tilth

Group II. Soils in the transition stage from group I to group III

Group III. Soils possessing a badly impaired physical condition

The data obtained have demonstrated that:

1. No regular relationship exists between field behavior and magnitude of saturation capacity provided it is greater than 0.25 per cent CaO.

2. The relationship between field behavior and the content of exchangeable calcium and the saturation deficit of the soil is markedly evident only in types of fairly uniform saturation capacity.

3. The soils of groups I, II, and III each lie within fairly well-defined zones of unsaturation.

Group I. 12 to 28 per cent, with a mean value of 21 per cent

Group II. 30 to 35 per cent, with a mean value of 32 per cent

Group III. Greater than 38 per cent

The delimiting values of the three zones appear independent of: the nature of the colloidal material of the soils examined; the degree of colloidity of the soil; any variation which may occur in the soil content of exchangeable ions other than calcium or hydrogen; the agricultural history of the soils.

The degree of unsaturation therefore appears to be a master factor controlling the field behavior of the soil. The relatively small amounts of organic matter present in the soils examined may aid in emphasizing its importance in this respect.

4. A general tendency exists for the pH value of the soil to decrease with increasing un-

saturation, but frequent and striking irregularities occur which indicate that other factors of primary importance contribute to the determination of pH value. In consequence, the soils of groups I, II, and III are not well defined by their hydrogen-ion concentration. The soil content of organic matter appears to be an important disturbing factor.

5. The modified procedure of Page and Williams gives much lower values than the titrimetric method of Hissink for the degree of unsaturation. A comparison of the values given by the two processes for a soil of low saturation capacity has afforded clear evidence that with the Hissink method reactions other than those of pure exchange occur.

III. Evidence is adduced that the soils of group I are in equilibrium with calcium carbonate. If such is the case, the saturation deficits of these soils are measures of the extent to which hydrolysis can occur, and the degree of unsaturation is a measure of the proportion of the acidoid material hydrolyzed under these conditions. This suggests that the degree of unsaturation of the good soils is a measure of the amount of calcium with which they can stably unite, rather than an indication of the point at which the ratio of exchangeable calcium to hydrogen is such that the former ion exerts a predominating influence over the properties of the soil, although in the good soils such a state of affairs may exist.

The limiting values found for the degree of unsaturation of the good soils (12 and 28 per cent) indicates that a considerable degree of variation with the soil occurs in the proportion of hydrolyzed material present under equilibrium conditions. This is probably due to variation in the strength of the acidoid material from soil to soil, a factor which may be influenced by the soil content and nature of humified organic matter.

A table has been given of the net amounts of calcium carbonate required to reduce to 20 per cent the degree of unsaturation of soils of varying saturation capacities and degrees of unsaturation.

Attention has been drawn to the need for maintaining a reserve of calcium carbonate in the soil, both for the purpose of repressing the ionization of the calcium salts of the acidoid material, and of preserving the degree of unsaturation at a value approximating to 20 per cent.

A comparison has been made of the lime requirements of a series of soils as determined by the method of unsaturation and the pH value method of Hardy and Lewis.

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THE DECOMPOSITION OF LIGNIFIED MATERIALS BY SOIL MICROÖRGANISMS

MAX. PHILLIPS, HERMAN D. WEIHE, AND N. R. SMITH¹

U. S. Department of Agriculture

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In examining the literature on the subject of the decomposition of lignin or lignified tissues by means of microörganisms, one gets the impression that lignin, either in the free state or in the chemical form found in various lignified plant tissues, is comparatively resistant to the action of microörganisms. The present investigation was undertaken to determine whether lignin as it occurs in plant materials can be decomposed by the microörganisms found in the soil.

Rose and Lisse (9) made a chemical examination of a sound sample of Douglas fir heart, also of one partly rotted, and of one almost completely rotted. They found that the percentage of cellulose and pentosans decreased with the advance in the decomposition of the wood, whereas the alkali-soluble fraction and the percentage of methoxyl increased. They conclude from their results that lignin is far more resistant to decomposition than cellulose, although no direct determinations of lignin were made.

Schrader (10) found that bacteria were incapable of breaking down lignin prepared by the Willstätter method, at least in the experimental period of 25 days.

Pringsheim and Fuchs (8), after adding the necessary inorganic salts, inoculated alkali lignin with forest soil. The product obtained differed from the original material in containing up to one-half of its weight of substances soluble in alcohol, although the original lignin was practically insoluble in alcohol. The alcohol-soluble fraction was found to have a smaller percentage of methoxyl but a greater percentage of carbon than the original lignin. The fraction insoluble in alcohol showed some decrease in percentage methoxyl, but the percentage carbon was not affected.

Bray and Andrews (2) and du Toit (14), studying the decomposition of wood with pure cultures of fungi and molds, found that the cellulose was rapidly decomposed, whereas the lignin remained practically intact.

Smith (13), in studying the chemical changes in apple wood brought about by decay due to *Polystictus versicolor* Fr., found that the percentage lignin was greater in the sample of decayed wood than in the sound wood. The absolute quantity of lignin present in each case was not determined directly. Using an indirect method, however, Smith obtains data which show that the lignin was practically unattacked.

Waksman (15) found that the lignins are more resistant to the action of fungi and bacteria than any of the other major ingredients of natural organic materials. The accumulation of lignin in the soil is believed by Waksman to account for a large part of soil humus.

Waksman and Tenney (16, p. 405) as a result of their studies on the decomposition of various plant materials conclude that, "Lignins are not decomposed in the soil, at least within the

¹ The two first-named authors are senior chemist and junior chemist, respectively, Color and Farm Waste Division, and the last-named author is senior bacteriologist, Division of Soil Microbiology, Bureau of Chemistry and Soils.

experimental period of 32 to 35 days. If they are decomposed at all, the amount of decomposition is only insignificant in comparison with the decomposition of the other constituents of natural organic matter."

Marcusson (7) determined the Cross and Beven cellulose and lignin in sound wood, in partly decayed wood, and in completely decayed wood. He found that when the material was first extracted on the water bath for 1 hour with 1 per cent sodium hydroxide solution and lignin determinations (fuming hydrochloric acid method) were made on this partly purified product, the percentage of lignin was about the same in all cases. The increase in alkali-soluble substances found by Rose and Lisse (9) in decayed wood was shown to be due to a degradation product of the cellulose. Waksman and Stevens (19) have shown, however, that the method used by Marcusson for the determination of lignin gives too low results because of partial removal of the lignin by the alkali.

Falck and Haag (4) conclude from their studies that in the microbiological decomposition of plant materials two distinct processes take place, namely, "destruction" and "corrosion." The effect of "destruction" is to decompose the cellulose and pentosans, the lignin being very little affected. "Corrosion" on the other hand causes slow decomposition of both lignin and cellulose.

Schwalbe and Ekenstam (11) examined pine wood which had been rotted by *Merulius lachrymans* and found the percentage of lignin very high, 73 per cent, whereas the percentage of cellulose was only 15 per cent. No pentosans were found. The lignin isolated from the rotted material by the Willstätter method was found to have a lower methoxyl content than that obtained by the same method from sound wood.

Waksman and Tenney (17, 18) studied the decomposition of the rye plant by soil microorganisms, as well as that of rye straw, cornstalks, and alfalfa tops. They found that the lignin decomposed less rapidly than any of the major plant constituents and conclude that (17, p. 332), "The accumulation of the lignins, which resist decomposition more than the other plant constituents, and the synthesis of microbial nitrogenous complexes account for the increase in soil 'humus' as a result of decomposition of natural organic materials."

EXPERIMENTAL METHOD

In the experiments here reported four typical lignified plant materials were used; namely, cornstalks, oat hulls, corncobs, and wheat straw. The materials were dried at 105° C. and the following determinations were made:

Pentosans were determined by Tollens' method. The directions given in the Book of Methods of the Association of Official Agricultural Chemists for carrying out this determination were followed (1).

Cellulose was determined by the Sieber and Walter (12) modification of the Cross and Bevan method. Instead of Gooch crucibles, prepared as recommended by Sieber and Walter, Jena sintered glass crucibles were used.

Lignin. The material was finely ground in a mill and the lignin determined by the fuming hydrochloric acid method of Willstätter and Zechmeister (2). The procedure recommended by Dore (3) was followed.

Ash was determined by igniting a weighed sample with a Bunsen burner and weighing the nonvolatile residue.

Methoxyl was determined according to the Kirpal and Bühn (6) modification of the Zeisel (5) method. To the hydriodic acid (10 cc.) 3 cc. phenol was added as recommended by Weishut (20).

Nitrogen was determined by the Kjeldahl method.

The results of the various determinations made on the original plant materials prior to their decomposition by the soil microorganisms are given in table I.

MICROBIOLOGICAL EXPERIMENTS

In order to determine whether the lignin present in cornstalks, oat hulls, corncobs, and wheat straw could be decomposed by the microorganisms of the soil, three series of experiments were conducted. In the first series, 10-gm. samples of the oven-dry (105°C.) materials were used and in the second and third series 25-gm. samples were employed. Czapek's solution was used as the culture medium in the first series of experiments (table 2). At the end of the incubation period of the first series of experiments, it was observed that the pH of the medium was rather high (pH 8.0 to 9.0). In the second and third series of experiments a modified Czapek's solution was accordingly employed. In place of the sodium nitrate an equivalent quantity of urea was added. When the modified Czapek's solution was used, the reaction of the medium remained close to neutrality throughout the experiment. The flasks containing the lignified plant materials were all inoculated with the same soil

TABLE 1
Composition of materials used in experiments with soil organisms
(Analysis made on materials dried at 105 °C.)

MATERIAL	PENTOSANS	CELLULOSE (CROSS AND BEVAN)	LIGNIN	ASH	-OCH ₃	N
	per cent	per cent	per cent	per cent	per cent	per cent
Corn stalks.....	24.51	52.4	16.75	5.28	2.60	1.08
Oat hulls.....	37.53	51.5	17.35	6.04	2.49	0.23
Corn cobs.....	36.18	56.2	23.90	1.47	2.23	0.36
Wheat straw.....	28.53	56.4	25.70	5.33	2.70	0.40

infusion and incubated at 28°C. The incubation period for the first series of experiments was from October 8 to November 13 (table 2) and for the second and third series, from December 20, 1928, to January 14, 1929, and from December 20, 1928, to February 20, 1929, respectively (tables 3 and 4). At the end of each incubation period the residual lignified material remaining in each flask was filtered off, washed with distilled water, dried at 105°C., and weighed. From the results thus obtained the loss in weight due to the microbiological action was determined. The percentages of pentosans, cellulose, lignin, ash, methoxyl, and nitrogen contained in this dry material were then determined by aforementioned analytical methods. The result of each determination was calculated on the basis of the initial weight of material used. By comparing these results with those obtained in the analysis of the materials in the original state, data were obtained showing the absolute loss of each constituent, brought about by the action of the microorganisms. The results of the first, second, and third series of experiments are recorded in tables 2, 3, and 4, respectively.

TABLE 2
Analytical results showing effect of selective decomposition of lignified materials with soil organisms
 (Analyses made on materials dried at 105°C.)

MATERIAL*	WEIGHT AT END OF EXPERIMENT		LOSS IN WEIGHT		PENTOSANS			CELLULOSE (CROSS AND BEVAN)			LIGNIN			ASH			METHOXYL			NITROGEN		
	gm.	per cent	Found	Calculated on original weight	Loss	Found	Calculated on original weight	Loss	Found	Calculated on original weight	Loss	Found	Calculated on original weight	Loss	Found	Calculated on original weight	Loss	Found	Calculated on original weight	Loss	Found	Calculated on original weight
Corn stalks.....	6.178	38.20	21.4	13.2	46.1	52.0	32.1	38.7	27.07	16.72	0.17	5.14	3.18	39.8	2.95	1.82	30.0	0.96	0.59	45.3	0.96	0.59
Oat hulls.....	8.770	12.30	38.4	33.7	10.2	52.5	46.0	10.6	18.72	16.41	5.4	5.55	4.86	19.5	2.71	2.38	4.4	0.16	0.14	39.1	0.16	0.14
Corn cobs.....	7.535	24.65	29.7	22.4	38.1	58.8	44.3	21.1	26.32	19.83	17.0	0.74	0.55	62.5	2.67	2.01	9.8	0.47	0.35	2.7	0.47	0.35
Wheat straw.....	6.983	30.17	26.5	18.5	35.0	53.1	37.1	34.2	22.15	15.46	39.8	4.52	3.85	27.7	3.78	2.64	2.2	0.46	0.32	20.0	0.46	0.32

* 10 gm. material used in each experiment. Incubated from October 8 to November 13, 1928.

TABLE 3
Analytical results showing the effect of selective decomposition of lignified materials with soil organisms
 (Analyses made on materials dried at 105°C.)

MATERIAL*	WEIGHT AT END OF EX- PERIMENT	LOSS IN WEIGHT			PENTOSANS			CELLULOSE (CROSS AND BEVAN)			LIGNIN			ASH			METHOXYL			NITROGEN		
		Found	Calculated on original weight	Loss	Found	Calculated on original weight	Loss	Found	Calculated on original weight	Loss	Found	Calculated on original weight	Loss	Found	Calculated on original weight	Loss	Found	Calculated on original weight	Loss	Found	Calculated on original weight	Loss
Corn stalks.....	13.80	44.8	25.54	14.10	40.7	46.9	25.9	50.5	22.46	12.40	25.9	2.27	1.25	76.1	3.35	1.85	28.8	1.20	0.66	38.8	0	
Oat hulls.....	21.55	13.8	40.90	35.25	6.0	47.7	41.1	20.2	18.50	15.94	8.1	5.23	4.50	25.5	2.74	2.36	5.2	0.28	0.24	0	0	
Corn cobs.....	19.27	22.9	30.40	23.43	35.2	50.3	38.7	31.1	18.28	14.09	41.0	0.58	0.44	70.0	2.84	2.18	2.2	0.50	0.38	0	0	
Wheat straw.....	17.07	31.3	28.74	19.62	31.2	52.7	36.0	36.1	19.44	13.27	48.3	4.28	2.92	45.2	3.50	2.39	11.4	0.46	0.31	22.5	0	

* 25 gm. material used in each experiment. Incubated from December 20, 1928, to January 14, 1929.

DISCUSSION

It will be observed from tables 2, 3, and 4 that in every case a considerable decrease in the quantity of pentosans and cellulose (Cross and Bevan) resulted from the action of the soil microorganisms. The absolute quantity of lignin, as well as the percentage of lignin, showed a decided decrease in nearly all of the experiments. In some of the experiments the loss of lignin, was as great as that of the cellulose and pentosans and in some instances even greater. This indicates conclusively that the soil microorganisms are capable of breaking down the lignin present in lignified plant materials. The greatest loss occurred in those experiments in which urea was used as the source of nitrogen. Whether by prolonged action the microorganisms of the soil are capable of breaking down all the lignin present, or whether a lignin degradation product is finally obtained which resists further decomposition, is not known. The experiments were not continued long enough to answer this question. Moreover, these experiments were conducted largely under aerobic conditions. Under strictly anaerobic conditions it may very well be, as some investigators hold, that the lignin is not decomposed, or at least only to a limited extent.

In all these experiments, except one, the methoxyl content decreased. Methoxyl groups are present in lignin, and with the loss of lignin a decrease in the methoxyl content of the material is to be expected. The authors are, of course, aware of the limitations of the methoxyl determination as applied to such a natural product as straw or cornstalks. That by far the largest percentage of the methoxyl found in lignified plant materials, such as straw, cornstalks, and corncobs, is due to the lignin present may be accepted as reasonably certain. The results on the methoxyl determinations also indicate a loss of lignin.

The fact that soil organisms are apparently capable of breaking down lignin as found in natural lignified plant materials in no way implies, of course, that they are equally capable of decomposing the lignin prepared either by the fuming hydrochloric acid method, by the 72 per cent sulphuric acid method, or by the alkali method. There is no evidence to support the claim that the lignin prepared in the laboratory is identical with the natural substance. The comparatively strong reagents that are employed for the isolation of lignin undoubtedly bring about drastic changes in its composition. It would hardly be expected that the lignin obtained by the hydrochloric, sulfuric, or alkali, methods would be identical with that in the plant substance.

SUMMARY

A study of the selective decomposition by soil microorganisms of the various constituents in cornstalks, oat hulls, corncobs, and wheat straw was made. The results indicate that a rapid decomposition of the pentosans and cellulose (Cross and Bevan) takes place.

Under proper conditions soil organisms are capable of decomposing lignin as found in lignified plant materials. Under suitable conditions the rate of

decomposition of the lignin may be as great as that of the cellulose (Cross and Bevan) and pentosans.

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THE DETERMINATION OF THE REPLACEABLE BASES AND THE BASE-EXCHANGE CAPACITY OF SOILS¹

H. D. CHAPMAN AND W. P. KELLEY

University of California

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The purpose of this paper is to present a brief discussion of the methods for, and the errors involved in, the determination of replaceable bases in soils; to discuss the results of some special studies on methods for the determination of replaceable bases in soils containing alkaline earth carbonates; and to give the results of special studies on the determination of base-exchange capacity by the NH_4 -absorption method.

The determination of the replaceable bases is complicated by the fact that various kinds of soil substances react with salt solutions and dilute acids. As is well known, the reaction which takes place between the base-exchange material and electrolytes is characterized by an exchange of ions, but with various other constituents this is not necessarily the case. Certain minerals that occur more or less widely in soils yield bases to salt solutions probably chiefly as a result of decomposition and ordinary solution processes. For example, finely ground hornblende, biotite, serpentine, and wollastonite are comparatively easily decomposed by neutral salt solutions, thus bringing into solution notable amounts of the bases of these minerals. In fact a number of the primary minerals that are commonly present in soils in varying stages of decomposition and alteration react to some extent with the reagents which are ordinarily used in the determination of the replaceable bases. It is well known that calcium carbonate is soluble in solutions of ammonium salts. When calcareous soils are being investigated, it is not exceptional to find that the calcium equivalent of the dissolved carbonate may equal or even exceed the replaceable calcium.

The importance of these incidental reactions, with the possible exception of those involving calcium carbonate, is apparently not always appreciated by students of base exchange, for it is common to report the data obtained by analysis of an extract of the soil as if all the calcium, magnesium, potassium, and sodium found had been replaced. Moreover, the treatment of the subject at the hands of various investigators often gives the impression that the determination of the replaceable bases is a clear-cut and precise process similar to the determination of total nitrogen or total calcium. As a matter of fact this is far from being true. When we consider that the nature of the equi-

¹ Paper No. 217, University of California, Graduate School of Tropical Agriculture and Citrus Experiment Station, Riverside, California.

librium between salt solutions and the exchange material makes it necessary to leach the soil for some time and with a considerable volume of the solution, that the salt solution inevitably dissolves or decomposes more or less of various minerals, that soils vary greatly in their leachability with the consequent variation in the length of time of contact between the salt solution and the soil, and that the true end point of the exchange reaction is often difficult to determine, it is evident that the determination of the replaceable bases is not a highly exact process. As a matter of fact sometimes special care is required to obtain good duplicate results with different samples of the same soil.

Ion exchange obviously denotes a reciprocal process. Upon treatment with a salt solution the soil not only gives up ions to the solution; it also absorbs an amount of base from the solution which is chemically equivalent to all of the ions that the base of the salt actually replaces from the soil. Substances which dissolve in the salt solution, or which react with the salt to form compounds that are unstable in the solution, will obviously yield quantities of base in excess of the amount that is absorbed. The extent to which solution and decomposition processes contribute to the results can be determined, however, by subtracting the sum of all cations that pass into the solution from the amount of that ion that is absorbed from the solution.

Failure to recognize that certain soils contain substances which undergo an exchange of their bases with barium, for example, but not with the alkalies, and that barium or calcium may be absorbed by soils from an alkaline solution, which elements may then be brought back into solution by the use of a neutral salt solution, but not necessarily as a result of ion exchange, has given rise to different methods for the determination of the replaceable bases, of replaceable H-ions, and of the base-exchange capacity of soils.

DETERMINATION OF REPLACEABLE BASES

Two types of method for the determination of the replaceable bases are in use at the present time. The method more commonly used involves the digestion and leaching of a weighed sample of the soil with a neutral salt solution or weak acid, the bases thus brought into solution being then determined. With the other method the replaceable bases are separated from the soil by electroanalysis and are determined in the dialysate.

Mattson (10), Bradfield (1), Wilson (17), and others have compared the amounts of bases found by means of neutral salt extraction and by electroanalysis, and have reported fairly good agreement with soils from the humid and have reported fairly good agreement with soils from the humid region. The results obtained in this laboratory are consistent with this conclusion. Since the presence of soluble salts, alkaline earth carbonates, and easily decomposable silicates affect the results obtained by electroanalysis as well as by neutral salt or weak acid extraction, not attention has been given in this study to the electroanalysis method.

Gedroiz (3) pointed out that with soils containing carbonate a determination

of the carbonate (CO_3) content of the soil both before and after neutral salt extraction affords a basis for calculating the extent to which calcium carbonate affects the results. Kelley and Brown (7) have found that this method is accurate if the soil does not also contain magnesium carbonate. Although Hissink (4) and MacIntire (9) have concluded that magnesium carbonate is nonexistent in humid soils, this is not the case with certain soils of semiarid regions. In fact it is possible that certain alkali soils contain both normal and basic carbonate of magnesium in addition to calcium carbonate. Inasmuch as all of these carbonates are soluble to some extent in ordinary salt solutions, the universal applicability of the Gedroiz correction is doubtful.

Hissink's method (4) for the determination of replaceable calcium in soils which contain calcium carbonate is as follows:

A 25-gm. sample of the soil is first digested for several hours at a temperature of about 70°C . with 250 cc. of N NaCl solution. The sample is then thrown on a filter and leached with fresh portions of the sodium chloride solution until two liters of leachate are obtained. The first liter of the leachate is kept separate from the second liter and calcium is determined in each liter separately. The calcium content of the first liter less that of the second liter is considered to be a measure of the replaceable calcium. This method involves the assumption that an equal amount of calcium carbonate is dissolved by each liter of the solution and that all of the replaceable calcium is removed by the first liter.

Burgess and Breazeale (2) proposed the use of $0.1\ N$ BaCl_2 solution for the determination of the replaceable bases of calcareous soils. If a large quantity of soil and a minimum of the barium chloride solution are used, they concluded that the replaceable calcium and magnesium can be determined accurately by this method. Burgess and Breazeale concluded, on the basis of special solubility studies, that unimportant amounts of calcium carbonate are dissolved by barium chloride under the conditions of their method. Tjurin (15) holds, however, that "considerable and inconstant" amounts of calcium are brought into solution by the interaction of barium chloride and calcium carbonate.

Recently Magistad and Burgess (10) suggested the use of an ethyl alcohol solution of barium chloride instead of an aqueous solution of this salt. The solubility of calcium carbonate in ethyl alcohol is lower than in water; hence the error in replaceable calcium caused by the solubility of calcium carbonate is decreased. They suggest that for accurate results a correction should be applied to the calcium found in the leachate on the basis of the theoretical solubility of calcium carbonate in this reagent.

According to Tjurin's method (15), 10 gm. of soil is leached with successive 500-cc. portions of N NaCl. The total content of carbonate and bicarbonate is determined in each portion of the leachate by titration with $0.02\ N$ HCl methyl orange being used as indicator. Calcium and magnesium are also determined in each portion. When the sum of the calcium and magnesium no longer exceeds the carbonate and bicarbonate content of the leachate, the replacement of the calcium and magnesium is considered to be complete. The

difference between the sum of the calcium and magnesium in the several portions of the leachate and the total carbonate and bicarbonate expressed as chemical equivalents, represents the sum of the replaceable calcium and magnesium.

Novak and Malac (12) and Hissink (6) have recently made comparative studies on the Hissink method and the ammonium chloride methods as outlined by Gedroiz (3) and by Kelley and Brown (7). The results were widely divergent. It seems probable that in these investigations no correction was made for the solution of calcium carbonate by ammonium chloride. If this be the case, then the conclusions concerning the relative accuracy of these methods are invalid.

In view of the limited and somewhat uncertain nature of previous studies on the various methods for the determination of replaceable calcium in calcareous

TABLE 1
Replaceable Ca as determined by different methods

METHOD	MILLIEQUIVALENTS PER 100 GM. SOIL	
	Ca	NH ₄ absorbed
NH ₄ Cl.....	0.00*	21.24
	0.00	21.24
Hissink.....	17.23
	17.01
Burgess and Breazeale.....	25.83
	26.08

* Calcium equivalent of carbonate dissolved exceeded the total calcium in leachate.

soils, it was decided to compare the ammonium chloride method, the Hissink method, and the Burgess and Breazeale method² on the carbonate-containing soil from California. The soil used is a dark-colored silt loam low in water-soluble constituents, and contains 6.9 per cent insoluble carbonate (CO₃). The methods as outlined by the original authors were followed as closely as possible. With the ammonium chloride method, total carbonate (CO₃) was determined both before and after the extraction and a correction was applied to the calcium data on the assumption that magnesium carbonate was non-existent in this soil. The data reported in table 1 reveal that this assumption was incorrect, for the equivalent of the dissolved carbonate exceeded the total calcium found; hence a part of the carbonate in this soil must be normal or basic magnesium carbonate.

The replaceable calcium found by the barium chloride method of Burgess

² When this part of the investigation was made the method proposed by Magistad and Burgess had not been published.

and Breazeale was somewhat in excess of the NH_4 absorbed from ammonium chloride. Whether this difference was occasioned by incomplete replacement of calcium and magnesium by NH_4 , or by errors that are inherent in the barium chloride method can not be stated definitely. Neither can the accuracy of the data obtained by the Hissink method be stated at present.

Special studies on the Hissink method were made to determine more definitely the approximate magnitude of the errors involved. For this purpose a neutral soil free from carbonate and low in water-soluble salts was used. Ten-gram samples of this soil, to which a known amount of calcium carbonate was added, were digested with 100 cc. of N NaCl at 70°C . over night. These solutions were then filtered and the soil was transferred quantitatively to a filter and leached with normal sodium chloride until 400 cc. of leachate was obtained. This amount of leaching is equivalent to 1,000 cc. for 25 gm. of soil. The leaching was then continued until another 400 cc. was obtained. Calcium

TABLE 2

Effect of CaCO_3 on the determination of replaceable Ca by the Hissink method (digested at 70°C .)

SAMPLE	MILLIEQUIVALENTS Ca PER 100 GM. SOIL		
	First 400 cc.	Second 400 cc.	Difference
Soil alone.	8.54	0.00	8.54
	8.40	0.00	8.50
Soil + $2\frac{1}{2}$ per cent CaCO_3	16.30	3.32	12.98
	16.60	3.12	13.48
	16.00	3.32	12.68
	16.70	3.32	13.38

was determined in both portions. Similar experiments were made with this soil without adding calcium carbonate. The results are recorded in table 2. They show that under the conditions of this experiment a greater amount of calcium carbonate was dissolved in the first than in the second portion of the leachate, as is indicated by the fact that the difference between the calcium content of the first and second portions is considerably greater than that found where no calcium carbonate was added.

Since the samples used in the foregoing experiment were first digested at an elevated temperature and then filtered and leached at ordinary laboratory temperature, as was recommended by Hissink, it seemed desirable to determine whether this preliminary digestion influenced the results. Accordingly another experiment was made with the same soil in which the preliminary digestion was made at room temperature. In all other respects the same technique was employed as in the previous experiment. The results are recorded in table 3. It will be noted that the temperature factor materially affected the solubility of calcium carbonate, but had no appreciable effect on the replacement of calcium.

By calculation it was found that the solubility of calcium carbonate was 0.098 gm. a liter in the first experiment (heated to 70°C.) and 0.062 gm. a liter in the second experiment (room temperature). Each of these quantities exceeds the theoretical solubility of calcium carbonate in normal sodium chloride solution. Without going into a theoretical discussion of this fact it seemed possible that different results might be obtained with soils which contain calcium carbonate naturally.

Accordingly samples of three different calcareous soils consisting of 25 gm. each were digested at 70°C. over night with 250 cc. of neutral *N* NaCl. The soil and solution were then quantitatively transferred to a filter and leached

TABLE 3

Effect of CaCO₃ on the determination of replaceable Ca by the Hissink method (room temperature)

SAMPLE	MILLIEQUIVALENTS Ca PER 100 GM. SOIL		
	First 400 cc.	Second 400 cc.	Difference
Soil alone.	8.40	0.00	8.40
Soil + 2½ per cent CaCO ₃	13.45	3.81	9.64

TABLE 4

The solution of CaCO₃ by normal NaCl under the conditions of the Hissink method

SOIL NUMBER	MILLIEQUIVALENTS PER 100 GM. SOIL		
	First liter	Second liter	
	CO ₃	CO ₃	Ca
709	1.84	1.32	1.28
709	1.96	1.32	1.28
534	2.72	2.76	3.44
534	2.64	2.76	3.28
8563	3.20	2.40	3.56
8563	3.24	2.28	3.16

until two liters of leachate had been obtained. The amounts of carbonate dissolved in the first and second liters, respectively, were determined by titrating 500 cc. with 0.1 *N* H₂SO₄, methyl orange being used as indicator. The calcium content of the second liter was also determined. The results, corrected for water-soluble carbonate, are shown in table 4.

It will be noted that the amount of carbonate found in the first liter of leachate from two of the soils is slightly in excess of that found in the second liter. If an elevated temperature had not been used in the preliminary digestion, it is probable that less carbonate would have appeared in the first liter of the leachate. With soils 534 and 8563 the second liter contained slightly more calcium than carbonate. It is probable, as was pointed out by Turner (16)

and Tjurin (15), that not all of the exchangeable calcium will be replaced by leaching 25 gm. of a highly calcareous soil with 1 l. of N NaCl solution.

The preceding data indicate that, should a greater amount of calcium carbonate be dissolved by the first liter of sodium chloride solution than by the second, the error thus introduced into the calcium data might be compensated for to some extent by the fact that not quite all of the exchangeable calcium is replaced by the first liter. Hence, upon subtracting the quantity of calcium found in the second liter from that in the first, approximately accurate figures for replaceable calcium may be obtained.

The next experiment was made with the alcoholic barium chloride method as proposed by Magistad and Burgess. Ten-gram samples of the same carbonate-free soil that was used in the experiments reported in table 2 were digested at room temperature with 200 cc. of the barium chloride reagent both with and without the addition of calcium carbonate. After occasional shaking for two hours the soil was transferred to a filter and leached until 800 cc. of leachate had

TABLE 5
Replaceable Ca as determined by the alcoholic-BaCl₂ method of Magistad and Burgess

SAMPLE	MILLIEQUIVALENTS Ca PER 100 GM. SOIL		
	First 400 cc.	Second 400 cc.	Difference
Soil alone.	8.34	0.00	8.34
	8.44	0.00	8.44
Soil + 2½ per cent CaCO ₃	9.88	1.25	8.63
	9.84	1.30	8.54
	9.78	1.35	8.43

been obtained. Both the first and second 400-cc. portions of the filtrate were analyzed for calcium by the method as outlined by Magistad and Burgess. The results are given in table 5. By subtracting the quantity of calcium found in the second 400 cc. of leachate from that of the first, approximately correct figures for replaceable calcium were obtained. These data indicate that by making a correction for the dissolved carbonate this method gives reasonably accurate results. Comparison of the data reported in tables 3 and 5 reveals that the solubility of calcium carbonate is considerably greater in an aqueous solution of sodium chloride than in alcoholic barium chloride.

Inasmuch as the method of Magistad and Burgess entails considerable analytical detail in the separation of calcium from barium, the use of an alcoholic solution of potassium chloride was suggested. Accordingly, 10-gm. samples of the soil used in the previous experiment, both with and without the addition of calcium carbonate, were digested at room temperature for one hour with 200 cc. of 0.2 N KCl dissolved in 63 per cent ethyl alcohol. The suspensions were then filtered and the soil was leached until 800 cc. of leachate

had been obtained. Calcium was determined in the first and second 400-cc. portions of the leachate. The results are given in table 6.

Comparison of the data reported in tables 5 and 6 shows that the solubility of calcium carbonate is slightly lower in alcoholic potassium chloride than in alcoholic barium chloride. Subtracting the quantity of calcium found in the second 400 cc. from that in the first, gives good agreement between the values.

The foregoing experiments suggest that approximately accurate determinations of replaceable calcium can be made in calcareous soil by several different methods, provided, however, that other easily decomposable or soluble calcium compounds are not present. With soils which contain easily soluble silicates of calcium or gypsum, no method is known to the writers by which it is possible to determine the replaceable calcium. Since calcium carbonate is only slightly soluble in alcoholic potassium chloride, it is possible that the silicates of calcium are also quite insoluble in this solution. If this be the case,

TABLE 6
Replaceable Ca as determined with alcoholic KCl

SAMPLE	MILLIEQUIVALENTS Ca PER 100 GM. SOIL		
	First 400 cc.	Second 400 cc.	Difference
Soil alone.....	8.30	0.00	8.30
	8.20	0.10	8.10
Soil + 2½ per cent CaCO ₃	9.40	1.07	8.33
	9.50	0.99	8.51

then this method would be applicable to all soils except those which contain gypsum.

By determining the replaceable potassium and sodium by the use of an ammonium salt, the replaceable calcium with alcoholic potassium chloride or barium chloride, and the total content of replaceable bases by the NH₄ absorption method (discussed more fully in the second part of this paper) it is possible to determine the replaceable magnesium by difference.

DETERMINATION OF BASE-EXCHANGE CAPACITY BY THE NH₄-ABSORPTION METHOD

"Base-exchange capacity," as we use the term, refers to the total quantity of cations which the soil is capable of holding in a form that is replaceable by, and stable in, neutral solutions of calcium, magnesium, potassium, sodium, and ammonium salts.

The Hissink (5) method for the determination of the base-exchange capacity first determines the replaceable bases and then determines the content of replaceable H ions by a special method. The sum of the quantities found, ex-

pressed as milliequivalents, represents the base-exchange capacity (T). Hissink's method (5) for determining the H ion involves the treatment of the soil with an excess of barium hydroxide. Hissink (6) holds that the replaceable bases are present in the soil as salts of weak acids and that the salts hydrolyze to some extent when brought into contact with water. Consequently these salts can be completely saturated with a strong base, such as calcium, only in regions of high alkalinity (pH 10 or more). This view may be theoretically sound. It seems to be of doubtful value in connection with soil investigations, however, for the base-exchange material probably neither was formed under conditions of high alkalinity, nor do such conditions exist in ordinary soils. Acid soils become alkaline when an amount of barium equivalent to Hissink's T -S values is added, but the same is true of colloidal silica and many other substances.

When we consider that, in addition to the base-exchange material, other soil substances, such as silica, the oxides of iron and aluminum, and various primary and secondary minerals, are able to absorb barium hydroxide, and the barium hydroxide thus absorbed is readily brought back into solution upon leaching with a neutral salt solution, it is evident that the determination of the barium that has been absorbed from barium hydroxide does not give reliable evidence as to the replaceability of the absorbed barium.

Several years ago Kelley and Brown (7) proposed the NH_4 -absorption method for the determination of the base-exchange capacity. According to this method the soil is first saturated with NH_4 and then the absorbed NH_4 is determined. Formerly ammonium chloride was used to saturate the soil with NH_4 . Since it is not possible to effect complete replacement of H ions³ by ordinary leaching with ammonium chloride, Kelley and Brown (8) later recommended the preliminary treatment of acid soils with an excess of barium hydroxide. Their method is based on the principle that all of the replaceable bases of a soil, whether it be completely saturated with base or only partially so, can be replaced by NH_4 .

A number of other methods for the determination of base-exchange capacity, either directly or indirectly, have been proposed, but inasmuch as the principles involved do not differ materially from those of the Hissink and the Kelley and Brown methods, they will not be discussed here.

The principles underlying the NH_4 -absorption method appear to be reasonably sound. The total NH_4 -absorbing capacity of a soil is a quantity which probably has as much agricultural significance as Hissink's (T) value, and, perhaps most important of all, this quantity gives a measure of total exchangeable ions, as ordinarily understood. Hence its determination makes it possible to distinguish between solution and decomposition processes, on the one hand, and replacement reactions, on the other.

³ The term "replaceable H ion," as used here and henceforth in this paper, refers to the quantity that is equivalent to the amount of barium hydroxide necessary to bring a soil to pH 7.0.

The advantages of ammonium salts over the other neutral salts that have been recommended are: NH_4 does not occur to any important extent in the natural soil; the absorbed and therefore replaceable NH_4 can be determined readily and accurately; NH_4 probably does not form insoluble compounds with components of the soil other than the exchange complex. Such substances, for example, as the addition compounds that are formed by an excess of calcium hydroxide or barium hydroxide, the precipitation products that may occur naturally or are readily formed artificially in alkali soils, and the intermediate and unstable products of weathering that occur in the relatively immature soils of semiarid regions, do not form insoluble compounds with NH_4 .

The results of special investigations with reference to the completeness of replacement of cations by NH_4 and the precise determination of the absorbed NH_4 are presented in the following pages.

Effect of time of digestion

Kelley and Brown (7) found that preliminary digestion of the soil with ammonium chloride at 70°C ., followed by leaching with ammonium chloride

TABLE 7

Effect of time of digestion on the amount of NH_4 absorbed from ammonium acetate

TIME OF DIGESTION	MILLIEQUIVALENTS NH_4 ABSORBED PER 100 GM. SOIL
20 minutes	26.25
15 hours	28.96
60 hours	29.72
72 hours	29.44

solution, brought about the absorption of a greater amount of NH_4 than mere leaching at room temperature. To determine the effect of more prolonged heating, 10-gm. samples of a neutral, carbonate-free soil, were digested with 250 cc. of neutral $N \text{CH}_3\text{COONH}_4$ (ammonium acetate) at 70°C . for periods of 20 minutes, 15, 60, and 72 hours. The soils were then filtered and leached until a volume of 400 cc. had been obtained. After removing the electrolyte by washing with methyl alcohol the absorbed NH_4 was determined by aeration.⁴ The results are reported in table 7. These data show that complete replacement was not effected when the digestion was limited to 20 minutes but became approximately complete within 15 hours.

Replacement of H ions

Inasmuch as complete replacement of H ions is not effected by ordinary leaching with ammonium chloride, Kelley and Brown (8) recommended the preliminary treatment of the soil with an excess of barium hydroxide as a

⁴ A modification of the well-known aeration method for the determination of NH_4 in soils has been worked out by A. P. Vanselow of this laboratory. This method has been used in all of the work reported in this paper.

means of replacing the H ions with barium, thus bringing the soil into a state of complete base saturation. Recently Schollenberger (14) suggested the use of ammonium acetate in place of ammonium chloride. Among other advantages, this salt will effect more complete removal of replaceable H ions than will ammonium chloride. That acetates will replace more H ions under ordinary leaching conditions than the corresponding chlorides is well known. Parker (13) has recently shown that the amounts of replaceable H ions, as determined by titrating acid soils to pH 7.0 with barium hydroxide and by the barium acetate method, are practically identical. His data suggest that complete replacement of H ions might be effected by leaching with neutral ammonium acetate solution.

In order to study this point, a comparison was made on a series of soils of the amounts of NH_4 absorbed from ammonium acetate both with and without preliminary treatment of the sample with barium hydroxide. With the barium

TABLE 8
 NH_4 absorbed from ammonium acetate with and without preliminary $\text{Ba}(\text{OH})_2$ treatment

SOIL	pH	MILLIEQUIVALENTS NH_4 ABSORBED PER 100 GM. SOIL	
		Without preliminary treatment	Treated with $\text{Ba}(\text{OH})_2$
431*	3.65	29.66	30.48
7576	4.77	61.45	62.11
3232	5.19	7.98	8.43
7526	5.30	46.71	46.74
11952	5.15	15.26	16.51
7092	7.41	15.93	15.77
7891	7.72	3.33	3.45
7575	5.80	108.90	115.15

* This sample had first been leached with 0.05 N HCl.

hydroxide treated series, 10-gm. samples were digested with 100 cc. of 0.1 N $\text{Ba}(\text{OH})_2$ for 24 hours. The soils were then filtered and taken up with 100 cc. of neutral N $\text{CH}_3\text{COONH}_4$. These suspensions were digested at 70°C. for 15 hours, filtered, and leached until the leachate was free from calcium and barium. The excess of ammonium acetate was washed out with methyl alcohol and the absorbed NH_4 was determined by aeration. The same general technique was used with the other series. The results are given in table 8. With the exception of a peaty soil, 7575, the results of the two series are in reasonably close agreement. They are in harmony with Parker's (13) data. Whether the slight increases in absorbed NH_4 incident to barium hydroxide treatment of the samples, were occasioned by the replacement of small quantities of H ions that were not replaced directly by NH_4 , or to the synthesis, under the influence of barium hydroxide, of a compound possessing base-exchange properties, or to some other factor, is not known. In any case it appears that

for all practical purposes neutral ammonium acetate will replace the H ions completely without preliminary treatment of the soil with barium hydroxide.

Effect of calcium carbonate on the absorption of NH_4

In view of the high solubility of calcium carbonate in ammonium acetate, it is possible that the calcium-ion concentration produced by the solution of calcium carbonate might be sufficient to prevent the complete replacement of exchangeable calcium by NH_4 . To test this point, different quantities of calcium acetate were added to a series of normal ammonium acetate solutions and these solutions were then employed in the determination using a carbonate-free soil. The results reported in table 9 show that a concentration of 10 p.p.m. calcium exerts no measurable influence on the absorption of NH_4 from a normal

TABLE 9
Influence of Ca ions on absorption of NH_4 by soils

Ca in SOLUTION	MILLIEQUIVALENTS NH_4 ABSORBED PER 100 GM. SOIL
p.p.m.	
0	28.96
5	28.70
10	29.22
500	26.44
1,000	25.90

TABLE 10
Influence of $CaCO_3$ on the absorption of NH_4

PER CENT $CaCO_3$ ADDED TO SOIL	MILLIEQUIVALENTS NH_4 ABSORBED PER 100 GM. SOIL
0.00	28.96
0.09	29.08
2.91	29.28
4.75	26.83
6.55	25.82

solution of ammonium acetate, but that concentrations of 500 and 1,000 p.p.m. do exert some effect.

In another experiment varying quantities of purified c.p. calcium carbonate were added to 10-gm. samples of a neutral carbonate-free soil. The samples were then treated with normal ammonium acetate in the usual way. The absorbed NH_4 is reported in table 10. These data demonstrate that calcium carbonate, if present in relatively large amount, may interfere with the replacement of calcium and therefore affect the accuracy of the results. In order to guard against this possibility the sample should be leached with ammonium acetate until the leachate is practically free from calcium.

Before making a direct determination of the absorbed NH_4 it is, of course, necessary to remove the occluded ammonium salts. For some time we have

leached out the ammonium salts with methyl alcohol, using the Nessler test to determine the end point. In connection with studies on this point it was found that the methyl alcohol leachate continued to give an appreciable test for NH_4 indefinitely. In an effort to determine whether this continued test for NH_4 represented traces of electrolyte still remaining in the soil or the loss of NH_4 split off from the exchange complex, the following study was made:

Six 10-gm. samples of a neutral soil were digested and leached with normal neutral ammonium chloride in the usual way. They were then washed with 200 cc. of double-distilled methyl alcohol. At this point the leachate gave only a faint test for NH_4 . Three of the samples were then washed with an additional 500 cc. of methyl alcohol. NH_4 was then determined in all the soil samples by the aeration method. NH_4 and chlorine determinations were also made in the last 500 cc. of washings.

The results are given in table 11. These data indicate fairly conclusively that NH_4 may be lost from the exchange complex by washing with an excess of methyl alcohol. Inasmuch as the alcohol leachings were perfectly clear,

TABLE 11
Loss of NH_4 from soil by prolonged washing with methyl alcohol

VOLUME OF METHYL ALCOHOL USED	MILLIEQUIVALENTS PER 100 GM. SOIL		
	NH_4 absorbed	NH_4 in last 500 cc. of washings	Chlorine in last 500 cc. of washings
cc.			
200	28.17
200	28.32
200	28.57
700	27.17	0.68	Trace
700	27.17	0.80	Trace
700	27.27	Trace

the loss of NH_4 was probably not due to the passage of colloidal material through the filter paper.

The methyl alcohol used in the foregoing experiment was found to be distinctly acid. Thus far we have been unable to obtain neutral methyl alcohol even by distillation over lime and sodium hydroxide. Apparently the available supply of this alcohol contains some aldehyde or ketone, which oxidizes to an acid in the vapor phase during distillation. When allowed to stand in contact with sodium hydroxide for several weeks, the alcohol became brown and viscous, indicative of the presence of aldehydes or ketones.

Further studies upon the losses of NH_4 occasioned by leaching with methyl alcohol strongly indicated that the losses noted in the last experiment were due to traces of acid in the alcohol.

To test this point further one quantity of redistilled methyl alcohol was adjusted to pH 7.0 with dilute ammonium hydroxide and another with sodium hydroxide. These solutions were then used in comparison with the original

methyl alcohol. The soil samples were first saturated with NH_4 by leaching with ammonium acetate solution and the occluded ammonium acetate was then displaced by leaching with neutral ammonium chloride. The electrolyte was removed by leaching with the aforementioned supplies of methyl alcohol, the chlorine test being used to determine the end point. The results are shown in table 12.

These data indicate that the loss of NH_4 , occasioned by the acidity of the methyl alcohol, can be overcome by neutralizing the alcohol with ammonium hydroxide. The data also indicate that it is not permissible to neutralize the

TABLE 12
Influence of pH of methyl alcohol upon the determination of the absorbed NH_4

pH OF ALCOHOL	VOLUME OF LEACHINGS	MILLIEQUIVALENTS NH_4 ABSORBED PER 100 GM. SOIL
	cc.	
5.2	300	28.9
7.0 (neutralized with NH_4OH)	220	31.3
7.0 (neutralized with NH_4OH)	300	31.9
7.0 (neutralized with NH_4OH)	500	31.5
7.0 (neutralized with NaOH)	400	28.0

TABLE 13
 NH_4 absorbed by soil as determined by making correction for the electrolyte left in the soil
Milliequivalents per 100 gm. soil

TOTAL NH_4	NH_4 AS NH_4Cl	DIFFERENCE = NH_4 ABSORBED
44.73	13.42	31.31
45.62	14.50	31.12
50.40	19.20	31.20

alcohol with sodium hydroxide, for the sodium ions thus introduced into the solution may replace a part of the absorbed NH_4 .

In order to eliminate the necessity for leaching with methyl alcohol, an experiment was made to determine the amount of electrolyte that is left in the soil after it has been saturated with NH_4 . Where ammonium chloride is used, determinations of NH_4 and chlorine in the sample make it possible to estimate the amount of NH_4 that is held in exchangeable form without the necessity of leaching out the electrolyte.

The samples, after treatment with the ammonium salt, were transferred directly to Kjeldahl flasks and NH_4 was determined by aeration. The amount found obviously included both the absorbed NH_4 and that present as ammo-

nium chloride. After the aeration was complete the contents of the flasks were acidified with nitric acid and chlorine determinations were made. By subtracting the NH_4 equivalent of the chlorine from the total NH_4 found, the absorbed NH_4 is obtained. The data are reported in table 13. The close agreement between the results obtained by this method and those obtained by washing out the electrolyte with methyl alcohol adjusted to pH 7.0 with ammonium hydroxide, indicates that either method may be used with confidence.

As has already been stated, the absorbed NH_4 was determined by the aeration method. Special experiments made to test the accuracy of this method have shown that it permits of practically complete recovery of the absorbed NH_4 . Under the conditions of this method the organic nitrogen compounds are not oxidized to NH_4 , whereas more or less oxidation may take place when distillation methods are used.

CONCLUSIONS

The determination of the replaceable bases of soils is not highly exact. The results are likely to be complicated by solubility and decomposition processes, which take place between the solutions used and various constituents of the soil. Such constituents are probably most important in relatively immature soils of dry climates.

The Hissink sodium chloride method, the alcoholic barium chloride method of Magistad and Burgess, and the method involving the use of alcoholic potassium chloride all give reasonably accurate results for the determination of replaceable calcium in calcareous soils, provided other soluble or decomposable calcium compounds are absent from the sample. If the soil contains such compounds it is probable that the replaceable calcium cannot be determined accurately by any method.

The base-exchange capacity of the soil can be determined by digesting and leaching the sample with normal ammonium acetate solution. This solution brings about approximately complete replacement of the H ions without the necessity of treating the sample with an excess of alkali. It was found that methyl alcohol is useful for the removal of the occluded electrolyte but that it is necessary to employ as nearly neutral methyl alcohol as possible. If the methyl alcohol is acid, its H ions may replace more or less of the absorbed NH_4 and thus introduce an appreciable error. The absorbed NH_4 can be accurately determined by aeration in the presence of sodium carbonate.

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CROSS INOCULATION WITH RHIZOBIUM RADICICOLUM

VICTOR BURKE AND NELSON J. HOHL

State College of Washington

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Attempts to modify the pathogenicity or symbiotic activity of *Rhizobium radicum* have generally met with failure. It has never been satisfactorily demonstrated that the root-nodule bacteria can be modified to grow on the rootlets of non-legumes. Cross inoculation between legume groups has been recorded but the possibility of extending the power of cross inoculation, if present, or of causing it to develop, if absent, beyond the natural limits of the strain of *Rhizobium radicum* has not been satisfactorily demonstrated (3). It is obvious that if cross inoculation between legumes cannot be induced or increased, it is useless to attempt inoculation of non-legumes with legume organisms.

In increasing the virulence of animal pathogens various procedures are followed. Growing the organism on the blood of the host, injecting the organism into tissues where it is less subject to the natural immunity of the host, or injecting massive doses to reduce the resistance of the host or exhaust its defensive mechanism, frequently results in the development of increased virulence. By these methods it is possible to cause non-pathogenic organisms to acquire some degree of pathogenicity, and pathogenic organisms which have lost their virulence to regain it. The degree of increase in pathogenicity is limited to a greater or less extent according to the species. Both the factors of adaptation and selection take part in the changes that occur. That *Rhizobium radicum* can be modified by exposure and selection has been demonstrated by Burke and Burkey (1).

Similar methods were applied by the authors in attempting cross inoculation between the different groups of legumes. It was also considered that if failure of cross inoculation depended on the inhibition of heterologous organisms by the plant juice this inhibitory factor might be utilized as a means of identifying strains.

EXPERIMENTAL

Nodule producing organisms were isolated from nodules on the rootlets of red clover (group 1), vetch (group 4), and alfalfa (group 2). To eliminate existing variations as an interfering factor, in making isolations from the nodules several typical colonies were selected and a composite stock culture was made from them. Each strain isolated produced nodules on the homologous but not on the heterologous legumes.

The legume seeds were inoculated and planted, and the plants were kept under observation for three months or longer in many cases. In some cases nodules were produced in 21 days. The method of growing the plants was that of Garman and Didlake (2). Uninoculated seeds were planted as controls. Each inoculation was in triplicate.

Experiment 1

The object of experiment 1 was to determine the effect of exposure to heterologous legume extract on the specificity of strains of *Rhizobium radicicolum*.

An alfalfa extract was prepared by grinding alfalfa roots in distilled water and sterilizing by filtering through a Berkefeld "W" filter. The filtrate was clear, yellowish, and transparent.

The vetch and red clover strains of *Rhizobium radicicolum* were grown on this medium for three months. Transplants were made every two weeks. The medium was favorable, as the organisms produced heavy growths. This favors the view that failure to produce nodules on heterologous legumes is not due to any inhibitory action of the plant juice. In order to avoid introducing any unnecessary changes, the plant juice used in this experiment was not sterilized by heat.

The vetch and red clover strains were next placed on alfalfa seeds and these planted and examined as previously described. As controls, vetch and red clover seeds were inoculated and planted as well as uninoculated seeds. The inoculated alfalfa seedlings failed to develop nodules. The vetch and red clover seedlings developed nodules. All other seedlings failed to develop nodules.

The results obtained favor the view that the nodule-forming character of *Rhizobium radicicolum* is highly group specific and not readily modified by exposure to the plant juice of a legume from another group. The experiment was repeated with various modifications in the technique used. The alfalfa root extract was added to equal parts of Ashby's medium and the organism grown on this. Extracts were made from the leaves and stems. Extracts from leaves, stems, and roots were sterilized by heat instead of by filtrations. In no case did prolonged growth on these extracts alter the nodule-production character of the vetch and red clover strains.

Since prolonged exposure to alfalfa extract failed to alter the specificity of red clover and vetch strains, these strains were exposed to living tissue. It was hoped that this experiment would determine whether the nodule-forming requirements of the strains can be modified by this method and whether failure to form nodules on heterologous legumes is due to the inhibitory action of the plant tissue.

Experiment 2

The object of experiment 2 was to determine the effect of exposure to living plant tissue on the specificity of strains of *Rhizobium radicicolum*.

Well-developed alfalfa plants with a stalk about one-half inch in diameter and without nodules were removed from the soil and washed thoroughly. The roots were sterilized in a 1:1,000 mercuric chloride solution for 15 minutes. They were then washed in several changes of sterile water and placed in flasks containing a nutrient solution. A cotton wrapping about the stalk at the mouth of the flask prevented contamination.

The nutrient solution remained sterile, indicating a satisfactory technique. The plants were now removed from the flasks and a "Λ" shaped cut was made through the outer layer of the root. The flap was loosened, a loopful of culture inserted, and the flap pressed into position. Two alfalfa plants were thus inoculated with the vetch strain and two with the red clover strain of nodule-forming organisms. The alfalfa plants were returned to the flasks and kept for three months. The "Λ"-shaped incision was above the nutrient solution, which remained sterile. The plants were then removed, the "Λ"-shaped flaps again loosened, and cultures made. In each case the strain inoculated was isolated in pure culture. Each strain was tested for nodule production of the homologous legume and on alfalfa. In each case the strain produced nodules on the homologous legume but not on alfalfa.

The results obtained indicate that specific nodule formation of strains of *Rhizobium radicum* is not readily modified by exposure to living plant tissue. This and the preceding experiment also indicate that failure to produce nodules is not due to any bactericidal action of the plant tissue. Bacteriostatic action may occur in the living tissue but since none occurred in fresh plant extract and the organism survived for a long time in living tissue, we must consider that such action, if present, is slight.

In our experiment, we attempted to modify only the nodule-producing organism. Since nodule formation depends on both the legume and the bacteria, positive results might be obtained by modifying both at the same time. The practical value of artificially modifying the legume plant is obscure. Natural modifications do occur in nature and the duplication of these experimentally to determine the possibility of cross inoculation is worthy of attention.

The possibility of separating the different strains of *Rhizobium radicum* by the rate of growth in legume extract was considered. Experiment 1 demonstrated that little, if any, bacteriostatic action for heterologous strains was present in legume extract. The following experiment was designed to determine this more accurately.

Experiment 3

The object of experiment 3 was to determine the comparative rate of growth of strains of *Rhizobium radicum* in homologous and heterologous legume extract.

Extracts of red clover, vetch, and alfalfa were prepared by macerating the roots in water and sterilizing by filtering through a Berkefeld "W" filter. A sample of each extract was inoculated with red clover, vetch, and alfalfa strains

of *Rhizobium radicicolum*. The cultures were examined after 12, 24, and 30 hours. No differences between the rate of growth in the homologous and heterologous extracts could be detected.

The red clover strain cultures showed definite growth in 12 hours, the alfalfa strain in 24 hours, and the vetch strain in 30 hours. Other cultures were obtained and tested for rate of growth to determine whether certain strains consistently grew more rapidly in legume extract. The results, given in table 1, are not definite. They indicate some variation in rate of growth between strains and between different cultures of the same strain. Whether this difference can be utilized to separate strains remains to be determined.

TABLE 1
Rate of growth of strains in legume extracts and in plain broth

STRAIN, BURRILL CLASSIFICATION	VETCH EXTRACT			RED CLOVER EXTRACT			ALFALFA EXTRACT			PLAIN BROTH		
	12 hours	24 hours	30 hours	12 hours	24 hours	30 hours	12 hours	24 hours	30 hours	12 hours	24 hours	30 hours
1*	x†	xx	...	x	xx	...	x	xx	...	x	xx	...
1 Wis.	x	xx	...	x	xx	...	x	xx	...	x	xx	...
1 W.S.C.	x	x	...	x	xx	...	x	xx	...	x	xx	...
2*	—	x	xx	—	x	xx	—	x	xx	—	x	xx
2*	x	xx	...	x	xx	...	x	xx	...	x	xx	...
2 Wis.	x	xx	...	x	xx	...	x	xx	...	x	xx	...
2 W.S.C.	—	—	x	—	—	x	—	—	x	—	—	x
4*	—	—	x	—	—	x	—	—	x	—	—	x
4 Wis.	—	x	x	—	x	x	—	x	x	—	—	x
4 W.S.C.	—	x	x	—	x	x	—	x	x	—	x	x
5 Wis.	—	—	x	—	—	x	—	—	x	—	—	—
5 W.S.C.	—	x	x	—	x	x	—	x	x	—	—	x
6 W.S.C.	—	x	x	—	x	x	—	x	x	—	x	x

* Isolated for these experiments. Wis. Obtained from the University of Wisconsin.
W.S.C. Washington State College laboratory cultures.

† x indicates faint growth; xx, heavy growth.

CONCLUSIONS

Legume tissue and extract do not have bactericidal action on heterologous strains of *Rhizobium radicicolum*. The extract alone does not have bacteriostatic action and probably the tissue does not.

Exposure to heterologous legume tissue and extract does not cause strains of *Rhizobium radicicolum* to change their specificity. Cross inoculation between certain of the legume groups is not made possible by this method. Whether the symbiotic activity of a strain for homologous legumes can be increased by exposure to plant juice or living plant tissue was not determined. It is probable that the limit of symbiotic activity of any strain depends upon exposure resulting in increased adaptation to the host and selection of the most active cells.

Different strains and different cultures of the same strain vary in their rate of growth in legume extract. The rate of growth in homologous and heterologous legume extract is the same. Whether the rate of growth of different strains varies sufficiently to be useful in identification is unknown.

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ELECTROFILTRATION: A NEW METHOD OF REMOVING EXCHANGEABLE BASES FROM SOIL COLLOIDS

AMAR NATH PURI

University Chemical Laboratory, Lahore, India

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The method of separating crystalloids from colloids by diffusion, accelerated by an electric current, is well known in colloid chemistry. The literature on the subject has been summed up by Humfeld and Alben (2). The application of this principle to soil problems is chiefly due to Mattson, whose work on electrodialyzed clays is well known. He has shown, for instance, that exchangeable bases in soils can be determined by this method, and the results obtained agree with those obtained by neutral salt displacement.

The Mattson cell consists of three compartments separated by a semipermeable membrane, such as parchment. The colloidal solution is placed in the middle section, and the outside ones are filled with distilled water. One of the outside sections contains a platinum gauze anode, and the other a copper or nickel cathode. When a potential difference of 20 to 200 volts is applied at these electrodes, the exchangeable bases collect as hydroxides in the cathode compartment, and can be determined by simple titration.

Bradfield, who confirmed Mattson's results (1), introduced a two-compartment cell consisting of an alundum extraction thimble, which is supported by a nickel cathode suspended in a specially constructed glass cell with a side arm. The platinum foil anode is placed on the inside of the alundum thimble. By a constant-level arrangement that maintains a flow of water, the exchangeable bases that appear in the cathode are continuously removed. The electro-dialysis is continued until the dialysate gives no coloration with phenolphthalein.

While working on similar lines, it appeared to the author that the technique could be further simplified to make possible the employment of the method for routine work in soil laboratories. With that object in view, two apparatuses, A and B, were devised and are described in the following. As will be seen later, they are based on a slightly different principle from the one hitherto employed, and for the sake of convenience of reference the name "Electrofiltration" is proposed.

APPARATUS A

Apparatus A is shown in figure 1. It consists of a glass cylinder (about 2.5 inches in diameter), a perforated copper disc provided with a screw for electrical connections, and a glass funnel provided with a flange, fitting exactly against

the flange of the cylinder. Attached to the perforated disc are two copper rings with diameter slightly greater than the flanges of the funnel and the cylinder, and in which the latter fit snugly. A filter paper (preferably Watman 50) is placed on the copper disc and the flange of the cylinder is dipped

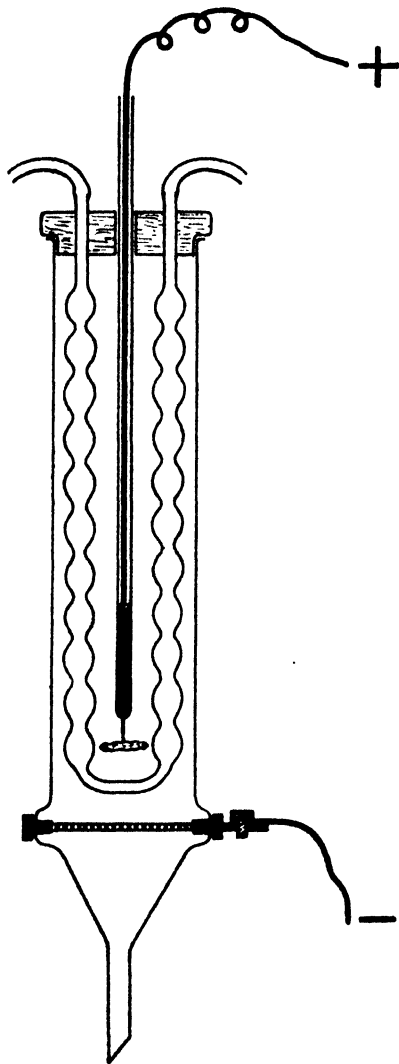


FIG. 1. DIAGRAM OF ELECTROFILTRATION APPARATUS A

into molten wax; after the excess wax is shaken off, the cylinder is attached to the disc with the filter paper interposed between. The flange of the funnel is also dipped into molten wax and attached to the other side of the disc. The flanges of the cylinder and funnel are then clamped together by brass rings provided with thumb screws (not shown in the diagram). Later it was found

preferable to have rubber washers attached to the flanges of the cylinder and the funnel.

The cathode is the copper disc, and the anode a platinum wire gauze. Attached to the wooden lid holding the anode is a U-tube for circulating cold water when the apparatus is running. The electrodes are connected to the electric main, and the current density is kept below 0.5 ampere by a lamp resistance. The current flowing through the apparatus is further regulated by moving the anode up or down as required. The apparatus can be left running overnight because when the level of water goes down below the anode the current is automatically shut off. Ten to twenty grams of the soil can be used at a time and the filtration is fairly rapid, for the tendency of the soil colloids is to move upward toward the anode, therefore there is no risk of the filter paper being clogged.

When the level of the water goes down, more is poured in, therefore a constant-level device was not found necessary. In fact the apparatus can be left to take care of itself after being started, and water filled in again when the first lot has more or less filtered through. The filtrate is collected all together or in several lots and titrated for total bases. It is better to back-titrate after adding excess of standard acid, as suggested by Bradfield. When the filtrate gives no color with phenolphthalein all the exchangeable bases have been removed. The anode is lifted along with the cooling device when the experiment is over. The apparatus can be dismantled, hot water being used for dislodging the wax, and can be reassembled in about 10 minutes.

It might be noted that there is no limit to the size of the apparatus. From a specimen jar 6 inches in diameter and with the base knocked off, the top of a vacuum desiccator, and a perforated brass disc having raised rims, an electro-filtration apparatus was assembled, with the help of rubber washers and suitable clamps, which worked quite satisfactorily, and could deal with about 500 gm. of soil at a time. Two U-tubes were used for cooling in this case.

APPARATUS B

Apparatus B, shown diagrammatically in figure 2, is much simpler, and very suitable for preparing soils free from exchangeable bases. It consists of a glass funnel and a perforated copper cone, which serves as the cathode. The anode is a platinum gauze (a platinum wire shaped into a coil serves equally well) attached to a glass tube, held in a wooden cover which also holds a V-shaped glass tube (not shown in the diagram) for circulating water. A filter paper is fitted into the copper cone as in ordinary filtration, and after the cone is filled with soil suspension the electrodes are connected with the electric main as in apparatus A.

Instead of a stout perforated cone, a nickel or copper gauze shaped like a cone can be used. A stout filter paper is preferable, but almost any brand can be used. The amount of soil that can be treated at a time depends on the size

of the apparatus, for which there is no limit. Sometimes the filtration is too rapid, and a stop-cock attached to the funnel is required to regulate the flow.

A battery of half a dozen electrofilters can be conveniently run, thus effecting a great saving of time for routine work.

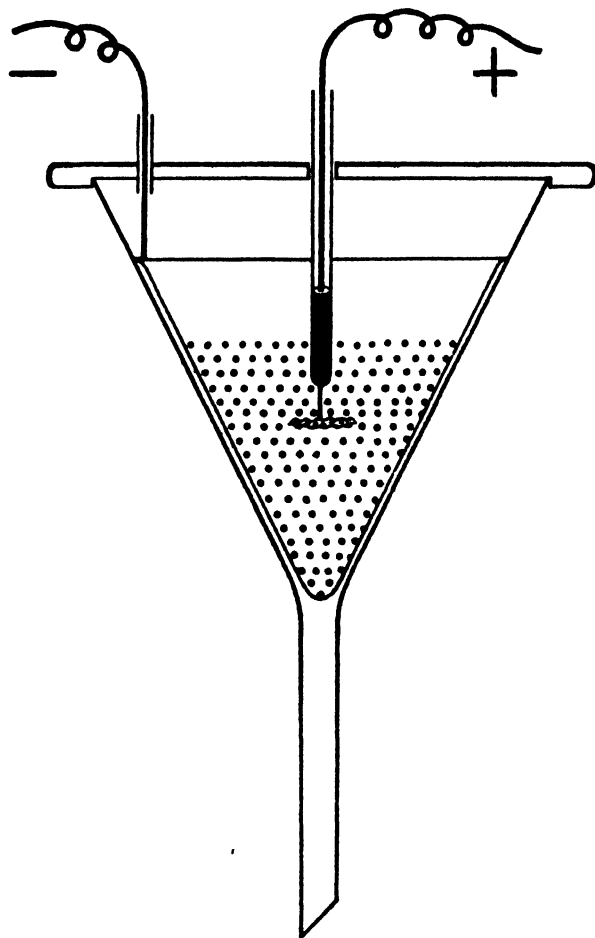


FIG. 2. DIAGRAM OF ELECTROFILTRATION APPARATUS B

ESTIMATION OF EXCHANGEABLE BASES IN SOILS

A very useful application of the electrofiltration method lies in the estimation of exchangeable bases in soils. It must be recognized, however, that only in the case of soils free from calcium carbonate, will the method give results identical to those obtained by neutral salt reaction. It is necessary that in the application of such methods we should differentiate between exchangeable bases and free bases present as carbonates. The removal of the latter by the neutral salt reaction methods, is purely a solubility effect, whereas in electro-

filtration or electrodialysis the reaction is electrochemical, and a greater amount of the carbonates is removed from the soil.

In table 1 are given the total bases removed in successive hours by electrofiltration (apparatus A), from a number of soils. The current was about 0.2 ampere and the weight of the soil 10 gm. in every case.

It will be seen from table 1 that the time required for the removal of bases by electrofiltration depends on the nature of the soil: soils 1 and 2 are calcarious and soil 6 is highly acid. The method as it stands, makes no distinction between exchangeable bases, and free bases present as carbonates. With this limitation, the electrofiltration method can be considered a rapid means of characterizing soils, and should afford some useful information regarding soil reaction.

TABLE 1
Bases removed by electrofiltration in successive hours from various soils

TIME <i>hours</i>	BASES (MILLIEQUIVALENTS) REMOVED					
	Soil 1	Soil 2	Soil 3	Soil 4	Soil 5	Soil 6
1	4 01	3 90	3 21	0 86	1 40	0.13
2	1 98	2 37	0 74	0 47	0 88	0 08
3	1 46	2 35	0 41	0 18	0 60	0.04
4	1 34	0 65	0 27	0 06	0.05	0.01
5	1 16	0 17	0.15	0.04	0
6	1 10	0 34	0.10
7	1 09	0 20	0 09
8	0 57	0 09	0 07
9	0 56	0 08
10	0 42
11	0 28

ELECTROFILTRATION AS A MEANS OF DISPERSING SOILS FOR MECHANICAL ANALYSIS

Both Mattson and Bradfield have drawn attention to the easy dispersibility of electrofiltered soil when treated with sodium hydroxide. A similar conclusion was reached by the author from his studies with soils treated with dilute hydrochloric acid; and it appeared of interest to compare the electrofiltration method with other preliminary treatment of the soil for mechanical analysis. Forty soils from different parts of India were used for comparison with three methods: the electrofiltration method, the (NaCl-NaOH) method developed by the author (3), and the 0.05 *N* HCl method of Puri and Amin (4).

In the electrofiltration method, the soil is electrofiltered for five or six hours, and then transferred with a jet of water to a stout beaker. Then 0.1 *N* NaOH is gradually added till the suspension is alkaline to phenolphthalein, tested by

TABLE 2
Mechanical analysis of soils by different preliminary treatments

SOIL NUMBER (P.C.)	MECHANICAL ANALYSIS				
	Silt (0.02 mm.)		Clay (0.002 mm.)		
	Electrofiltration method	(NaCl-NaOH) method	Electrofiltration method	(NaCl-NaOH) method	0.05 N HCl method
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
8	3.5	7.0	27.2	25.2	23.9
9	15.7	14.8	22.7	21.6	14.6
10	11.0	14.5	36.5	35.6	35.6
11	11.6	11.1	31.4	32.8	30.6
12	16.1	20.5	6.0	3.8	7.2
13	20.4	19.6	56.4	58.9	56.7
14	23.7	23.8	16.0	22.3	21.5
15	21.4	26.2	20.5	21.9	22.0
16	15.1	16.5	9.6	7.3	8.7
17	23.4	23.5	14.7	14.2	14.1
18	62.8	61.4	20.6	22.2	22.6
19	17.4	19.6	41.5	42.4	40.7
20	9.4	9.2	8.1	6.5	8.1
21	26.1	28.9	16.3	13.5	14.6
22	17.2	16.3	14.5	15.2	13.9
23	13.1	13.8	10.8	11.3	11.1
24	14.4	21.2	14.0	8.0	9.7
25	4.1	2.2	3.7	4.0	3.7
26	5.3	4.2	23.3	22.6	23.5
27	21.1	16.3	42.3	53.2	51.1
28	15.9	21.3	48.7	44.6	43.0
29	18.4	21.3	58.1	63.0	61.2
30	18.6	21.1	52.4	54.1	52.0
31	7.1	10.2	24.7	22.8	22.6
32	24.4	20.8	62.5	62.9	64.6
34	41.5	42.9	13.1	11.3	12.5
35	19.4	20.9	18.9	18.3	19.8
36	13.1	11.9	12.3	11.7	12.0
37	21.8	21.1	9.2	4.1	5.9
38	18.9	20.0	51.4	52.9	51.2
39	10.5	9.5	10.4	8.5	8.9
40	6.7	6.5	12.6	13.1	13.0
41	17.0	22.8	50.6	53.4	51.4
42	19.1	22.9	50.6	53.4	52.8
43	38.1	36.5	11.6	19.7	21.6
44	20.3	19.2	8.8	8.4	9.1
45	17.9	18.0	11.8	10.7	11.1
46	17.6	16.6	55.2	56.4	54.0
47	29.4	32.7	16.7	17.1	16.4
48	27.3	30.8	19.8	19.8	19.6

taking a drop on a tile or by throwing a drop of the indicator on the suspension. No shaking is necessary but the suspension is occasionally stirred during the course of six hours.

In the 0.05 *N* HCl method the soil was washed with 0.05 *N* HCl till the filtrate was free from Ca ions, 0.1 *N* NaOH then being added exactly as above.

In the (NaCl-NaOH) method, the soil was washed with *N* NaCl followed by leaching with only a small quantity of water. Sodium hydroxide was then added to make the suspension alkaline to phenolphthalein, as stated in the foregoing.

The results given in table 2 show that all three methods give closely agreeing values for clay, and the choice of any one of them may be a matter of personal inclination.

The mechanical analyses were done by the pipette method, the technique described by Puri and Amin (4) being used. All values refer to air-dry soils.

SUMMARY

Two forms of electrofiltration apparatus for removing exchangeable bases from soil colloids have been described.

Electrofiltration can be used as a preliminary treatment of the soil for mechanical analysis, and gives closely agreeing values for clay with the (NaCl-NaOH) method and the 0.05 *N* HCl method, which have already been shown to effect complete dispersion of soil colloids.

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RELATION OF ASH CONSTITUENTS OF PASTURE PLANTS TO THE OXIDATION-REDUCTION POTENTIALS OF NUTRIENTS

H. P. COOPER AND J. K. WILSON

Cornell University

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The lack of a close correlation between the hydrogen-ion concentration of soils and the growth of plants leads to a consideration of the electrical potential relations which may result from the presence in the soil of ions above and below hydrogen in the electromotive series. Since many organisms are known to absorb selectively, metallic nutrient ions in the same qualitative order as they appear in the electromotive series, it appeared desirable to determine the ash constituents of plants representing the pasture plant associations characteristic of the various soil fertility levels found in New York pastures. These associations and their ecological significance are described in previous papers (6, 8). A more or less characteristic succession of pasture plants accompanies the depletion of such soils. The particular species which dominates at the various fertility levels is often determined by climatic conditions. Kentucky bluegrass which requires a fertile soil may be followed by redtop in relatively dry climates or by Rhode Island bent grass in cooler climates with relatively high growing-season rainfall.

Kentucky bluegrass requires a fertile soil, and it is likely to dominate on the productive soils. As the soil is depleted, Rhode Island bent grass or redtop encroaches upon the Kentucky bluegrass and may finally dominate. On further depletion of the soil sweet vernal grass may dominate, and finally poverty grass, weeds, and trees represent the dominant type of vegetation (8).

SELECTION OF MATERIAL FOR ANALYSIS

The samples were taken when the grasses were in full bloom or shortly thereafter. The samples of Kentucky blue and sweet vernal grasses were collected during June and early July. The Rhode Island bent and poverty grasses were obtained three or four weeks later, since they do not bloom as early as do the Kentucky blue and sweet vernal grasses. When the samples of grass were taken the soils on which they were growing were sampled and their hydrogen-ion concentrations determined potentiometrically.

Most of the Kentucky bluegrass samples were taken from Ontario loam, which is a limestone soil. Many of the other samples were taken from non-limestone soils. The Rhode Island bent, sweet vernal, and poverty grass samples were collected from the Wooster, Voiusia, and Gloucester series. The broomsedge sample was from Merrimac sandy loam.

TABLE 1

Analyses of pasture grasses representing the various plant associations characteristic of the different soil fertility levels found in New York pastures

NUMBER OF SAMPLE	pH OF		PER CENT OF DRY MATTER						
	Soil	Subsoil	K ₂ O	CaO	MgO	P ₂ O ₅	SiO ₂	N	Ash
<i>Kentucky bluegrass—Poa pratensis</i>									
1	7.84	7.63	2.77	0.14	0.18	0.54	3.87	1.56	7.52
2	6.99	6.83	2.42	0.16	0.18	0.57	1.63	1.53	6.12
3	6.91	1.65	0.27	0.18	0.47	1.05	1.14	4.68
4	6.70	1.79	0.31	0.21	0.47	0.77	1.48	4.61
5	7.79	1.79	0.24	0.18	0.42	1.11	1.21	4.41
6	6.85	6.80	3.02	0.11	0.22	0.71	3.77	1.79	8.23
7	6.64	6.83	2.84	0.14	0.16	0.74	1.69	1.91	6.79
8	5.51	5.64	2.62	0.18	0.19	0.76	1.38	1.79	6.26
9	5.92	5.97	2.33	0.08	0.13	0.49	1.89	1.19	5.70
Average...	6.79	6.62	2.36	0.18	0.18	0.57	1.91	1.51	6.04
Per cent of ash.....			39.07	2.98	2.98	9.44	31.62	25.00	
<i>Bent grasses—Agrostis tenuis and Agrostis canina</i>									
1	5.19	5.31	2.08	0.34	0.16	0.39	1.10	1.49	5.18
2	4.48	4.67	2.16	0.19	0.14	0.64	2.17	1.40	6.30
3	5.75	5.76	1.45	0.38	0.21	0.34	3.03	1.47	6.11
4	5.18	5.25	0.78	0.43	0.32	0.32	2.76	1.35	5.09
5	5.15	5.15	2.34	0.41	0.21	0.47	1.81	1.46	5.90
6	5.30	4.81	1.68	0.38	0.19	0.34	2.59	1.16	5.77
7	4.80	4.69	1.90	0.36	0.23	0.39	1.44	1.26	4.95
8	4.90	1.61	0.36	0.19	0.39	2.01	1.17	5.27
9	4.87	4.30	1.50	0.33	0.16	0.34	2.99	1.26	5.73
10	4.30	5.40	1.75	0.44	0.16	0.39	2.79	1.42	6.28
11	7.51	7.51	0.85	0.23	0.11	0.39	4.10	1.64	6.29
12	5.08	5.23	0.39	0.25	0.21	0.32	3.57	1.07	5.23
13	5.02	5.40	0.48	0.21	0.13	0.32	2.88	0.99	4.43
Average...	5.19	5.29	1.46	0.33	0.19	0.39	2.56	1.32	5.58
Per cent of ash.....			26.16	5.91	3.40	6.99	45.88	23.65	
<i>Sweet vernal—Anthranthum odoratum</i>									
1	5.38	5.42	1.74	0.19	0.13	0.35	1.06	1.13	4.23
2	5.22	5.76	1.88	0.22	0.13	0.32	1.00	1.13	4.33
3	5.22	5.34	2.14	0.11	0.13	0.52	1.31	1.23	5.02
4	5.92	5.97	2.04	0.12	0.13	0.44	1.25	1.10	4.89
5	5.35	5.48	1.56	0.18	0.13	0.40	1.02	0.95	3.92
6	4.61	4.67	1.70	0.14	0.11	0.42	1.25	1.13	4.21
Average...	5.28	5.44	1.84	0.16	0.13	0.41	1.15	1.11	4.43
Per cent of ash.....			41.53	3.61	2.93	9.25	25.96	25.06	

TABLE 1—*Concluded*

NUMBER OF SAMPLE	pH of		PER CENT OF DRY MATTER						
	Soil	Subsoil	K ₂ O	CaO	MgO	P ₂ O ₅	SiO ₂	N	Ash
<i>Poverty grass—Danthonia spicata</i>									
1	4.61	5.31	1.24	0.27	0.13	0.17	1.77	0.67	3.99
2	6.87	5.68	1.43	0.27	0.11	0.17	0.68	0.64	3.27
3	5.97	5.80	1.37	0.28	0.13	0.22	1.13	0.99	3.44
4	4.95	5.05	1.74	0.25	0.14	0.28	1.44	1.02	4.29
5	5.55	5.48	1.47	0.26	0.16	0.26	0.78	1.10	3.47
Average...	5.59	5.46	1.45	0.27	0.13	0.22	1.16	0.88	3.69
Per cent of ash.....			39.29	7.32	3.52	5.96	31.44	23.85	
<i>Broomsedge—Andropogon virginicus</i>									
1	4.74	5.04	0.40	0.19	0.05	0.30	4.21	0.71	5.14
Per cent of ash.....			7.78	3.70	0.97	5.84	81.91	13.81	

ASH CONSTITUENTS OF THE GRASSES COLLECTED

The chemical composition of certain of the grasses which were collected are shown in table 1. The figures represent the composition of those plants which compose the most common plant successions accompanying the depletion of soils in New York pastures.

The nitrogen and the silica-free ash contents of these plants seem to decrease as the soils become depleted. Potassium, reported as the oxide, constitutes from about one-third to two-fifths of the ash content of common pasture plants. It is relatively high in Kentucky blue and sweet vernal grasses, whereas it is relatively low in Rhode Island bent and poverty grasses. The Rhode Island bent and poverty grasses contain the largest quantities of calcium. This inverse relationship between the content of potassium and calcium in the different grasses seems to be related to the grand growth period of the species. Plants, such as Kentucky blue and sweet vernal grasses, with their grand growth period early in the season are relatively high in potassium and low in calcium, whereas the species with their grand growth period coming relatively late in the season are higher in calcium and lower in potassium.

A negative correlation of -0.87 ± 0.023 was found between the K₂O and CaO content in the ash of 45 samples of pasture plants (6). An average of 0.18 per cent of CaO was found in the ash of Kentucky blue and 0.33 per cent in the ash of Rhode Island bent grass. This is an interesting relationship, since the Kentucky bluegrass samples were taken from limestone soil. The average pH value of 6.79 given in table 1 indicates that the soils were well supplied with available calcium. Most of the Rhode Island bent samples were taken from non-limestone soils. The average pH value of the soils on

which the Rhode Island bent grass was found is 5.19. The inverse relationship between the quantities of potassium and calcium in the plants analyzed would seem to indicate that the ash constituents of plants are largely determined by the amounts of these constituents which are available in the soil. These data are in agreement with the growth response of Kentucky bluegrass to potassium fertilization, as reported by White and Holben (26) and by White and Gardner (27).

TABLE 2
Analyses of grasses found in some New York pastures

NUMBER OF SAMPLE	pH OF		PER CENT OF DRY MATTER						
	Soil	Subsoil	K ₂ O	CaO	MgO	P ₂ O ₅	SiO ₂	N	Ash
<i>Canadian bluegrass—Poa compressa</i>									
1	7.80	1.58	0.21	0.11	0.34	0.46	1.05	3.56
2	7.63	7.63	1.08	0.33	0.13	0.17	3.07	0.68	5.14
3	7.57	1.06	0.23	0.16	0.17	0.80	0.79	2.64
4	7.57	0.99	0.34	0.18	0.22	1.02	0.78	3.10
Average...	7.64	7.63	1.18	0.28	0.15	0.23	1.34	0.83	3.61
Per cent of ash.....			32.69	7.76	4.15	6.37	37.12	22.99	
<i>Orchard grass—Dactylis glomerata</i>									
1	7.58	2.90	0.19	0.16	0.71	1.66	1.23	6.84
2	7.47	7.35	4.71	0.11	0.20	0.87	2.65	2.10	10.16
Per cent of ash.....			44.70	1.76	2.12	9.41	25.29	19.53	
<i>Rice cut grass—Leersia oryzoides</i>									
1	5.09	0.49	0.19	0.13	0.36	9.29	0.97	10.93
2	5.26	0.53	0.23	0.21	0.50	7.02	1.40	9.07
Average...	5.17	0.51	0.21	0.17	0.43	8.15	1.18	10.00
Per cent of ash.....			5.10	2.10	1.70	4.30	81.50	11.80	

The average quantities of phosphorous, reported as P₂O₅, in the ash of Kentucky bluegrass and sweet vernal grass is relatively high; on the other hand, the phosphorus content of the Rhode Island bent and poverty grasses is relatively low. This appears to be a significant relationship. The phosphorous content is positively correlated with potassium and negatively correlated with calcium in 45 samples (6). A positive correlation of $+0.76 \pm 0.043$ was observed between K₂O and P₂O₅ in the ash of plants, and a negative correlation of -0.44 ± 0.08 between the CaO and P₂O₅. It is probable that under certain conditions both plants and animals can assimilate the phosphorous of potassium phosphates more readily than that of calcium phosphates.

The potassium phosphates are more soluble than calcium phosphates, and they have a much higher ionization coefficient, which probably facilitates the reduction of the phosphate ion to a phosphite ion. The ease with which phosphorus is assimilated is probably dependent upon the reduction of the phosphate ion either by radiant energy, as from the sun, or by the free energy decrease in oxidation-reduction reactions (6).

There is a negative correlation between the P_2O_5 and SiO_2 in the ash of the plants studied (6). The sample of broomsedge was found to be very high in silicon.

Certain of the ash constituents of Canadian blue, orchard, and rice cut grasses are shown in table 2. Both the total ash and the nitrogen content of the Canadian bluegrass samples are low. This species probably tolerates a lower fertility level than does Kentucky bluegrass. The rice cut grass is very high in silicon. It is probable that silicic acid or other silicates is playing a rôle in the nutrition of both rice cut grass and broomsedge.

A summary of the analytical data is given in table 3. It is observed that the nitrogen and the silica-free ash tend to decrease as the soils are depleted. The nitrogen ranges from 1.51 per cent in Kentucky bluegrass to 0.71 per cent in broomsedge. The average contents of K_2O , CaO and MgO in the plants are 1.33 per cent, 0.22 per cent and 0.14 per cent respectively, which is in agreement with the order of these elements in the potential series. These data demonstrate the correlation between the order of electropositive elements in the electromotive series and their relative amounts in the ash of plants.

REMOVAL OF ASH CONSTITUENTS OF PLANTS FROM SOIL COLLOIDAL COMPLEXES AND ABSORPTION OF NUTRIENT IONS

Since materials with relatively high standard electrode potentials, such as potassium or calcium, are often the predominant metallic constituents of plants, it is interesting to note the qualitative order of removal of cations from soil colloidal complexes by electrodialysis. Koenig, et al. (15) noted that some of the potassium is easily removed from the soil. Their results from the electrodialysis of six soils show that the amount of potassium removed was more than five times that of the magnesium. Recent data reported by Mattson (16, 17, 18) and Wilson (28) may be interpreted to show that there is a correlation between the qualitative order of removal of metallic exchangeable atomic cations from soil colloidal complexes by electrodialysis and their arrangement in the electromotive series. The work of Gedroiz (13) on base exchange shows a similar relationship.

The order of removal of atomic cations from soil colloidal complexes is also probably closely correlated with the ionization potentials of elements. The approximate energy of removal, in equivalent volts, of the inmost normal valence electron of elements to form ions is as follows: Cs^+ 3.87 volts, Rb^+ 4.15 volts, K^+ 4.30 volts, Na^+ 5.13 volts, Li^+ 5.40 volts, Ba^{++} 9.96 volts, Sr^{++} 10.98 volts, Ca^{++} 11.82 volts, H^+ 13.54 volts, Mg^{++} 14.97 volts, and

Al⁺⁺⁺ 28.32 volts (9, 12, 22). There is probably a closer agreement between the order of removal of atomic cations from exchange complexes of soil by electrodialysis and the preceding grouping than there is with the electromotive series or displacement grouping of elements.

The foregoing values suggest an interpretation for the relatively large amounts of hydrogen ions in certain soil colloidal complexes. Hydrogen comes between calcium and magnesium in the foregoing grouping. There is usually a relatively small amount of exchangeable magnesium in the colloidal complex of most non-saline soils. Such elements as aluminum with an energy

TABLE 3

Nutrient elements in the ash of pasture grasses representing the various plant associations characteristic of the different soil fertility levels found in New York

GRASS	NUMBER OF SAMPLES	AVERAGE pH	K ₂ O	CaO	MgO	P ₂ O ₅	SiO ₂	N	ASH
<i>Average per cent of dry weight</i>									
Kentucky blue.....	9	6.79	2.36	0.18	0.18	0.57	1.91	1.51	6.04
Rhode Island bent.....	12	5.19	1.46	0.33	0.19	0.39	2.56	1.32	5.58
Sweet vernal.....	6	5.28	1.84	0.16	0.13	0.41	1.15	1.11	4.43
Poverty.....	5	5.59	1.45	0.27	0.13	0.22	1.16	0.88	3.69
Broomsedge.....	1	4.74	0.40	0.19	0.05	0.30	4.21	0.71	5.14
Rice cut.....	2	5.17	0.51	0.21	0.17	0.43	8.15	1.18	10.00
Average.....			1.33	0.22	0.14	0.39	3.19	1.12	5.81
<i>Average per cent of the ash of plants</i>									
Kentucky blue.....	9	6.79	39.07	2.98	2.98	9.44	31.62	25.00	
Rhode Island bent.....	12	5.19	26.16	5.91	3.40	6.99	45.88	23.65	
Sweet vernal.....	6	5.28	41.53	3.61	2.93	9.25	25.96	25.06	
Poverty.....	5	5.59	39.29	7.32	3.52	5.96	31.44	23.85	
Broomsedge.....	1	4.74	7.78	3.70	0.97	5.84	81.91	13.81	
Rice cut.....	2	5.17	5.10	2.10	1.70	4.30	81.50	11.80	
Average.....			26.49	4.27	2.58	6.96	49.72	20.53	

of removal of the inmost normal valence electron of 28.32 equivalent volts would be expected to form complex ions in most non-saline soils.

Data on ash analysis of 48 plants reported by Robinson, et al. (23) show the order of mineral constituents as follows: K₂O 34.36 per cent, CaO 19.63 per cent, MgO 7.13 per cent, Al₂O₃ 1.34 per cent, and Fe₂O₃ 0.61 per cent. Aston (2, 3, 4) reports data on the constituents of 70 samples of pasture plants, which include samples from regions of New Zealand where iron is deficient in forage plants. His average data for 47 to 70 samples are as follows: CaO 1.05 per cent, MgO 0.47 per cent, Al₂O₃ 0.13 per cent, Mn₂O₃ 0.045 per cent, and Fe₂O₃ 0.041 per cent. These data are in general agreement with the analyses

reported in this paper. All of these data show that many plants selectively absorb the strong ions or the elements with relatively high standard electrode potentials.

RELATION OF SOIL FERTILITY AND LIGHT REQUIREMENTS OF PLANTS TO THEIR FOOD VALUE

A book by Orr (22) contains an excellent summary of data on the mineral content of pasture plants, therefore only recent data of particular interest are cited in this paper. The relation to nutrition of electrochemical factors which are of interest in this connection have been discussed in previous papers (6, 7, 8). The differential absorption of the stronger ions may limit or exclude essential but weaker nutrient materials such as magnesium, manganese, and iron. Jones (14) found that a deficiency of available magnesium in the soil was responsible for chlorosis of certain crops. Magnesium is apparently the most electropositive element in the chlorophyll complex.

The common chemical fertilizers usually contain materials which form strong ions, therefore an application of fertilizer may very materially affect the chemical composition of plants. The affect of nitrogenous fertilizer in increasing the nitrogen content of the plant has been reported by Enlow and Coleman (11). Data reported by Archibald and Nelson (1) and by Ellenberger, et al. (10) show that applications of chemical fertilizer increased the yield and modified the composition of pasture plants. Brown and Slate (5) have reported on the growth response of pasture plants to the various fertilizer constituents.

The available data on both growth response and chemical analysis indicate that nutrient materials which form strong ions are usually the factors limiting the production of pastures in humid climates (6, 7, 8). The fertility of the soil greatly affects the quality as well as the total production of pastures. A discussion of the rôle of pastures in the mineral nutrition of farm animals by Maynard (20) is of interest in this connection.

The available data suggest that plants which require fertile soils and strong ions for their optimum growth also require light of high quality. Many plants which grow normally on poor soil and endure weak nutrient ions are often tolerant of shade and are of low food value.

The absorption of radiant energy of quality equal to or greater than the free energy decrease in the formation of simple electrolytes from elements greatly facilitates their assimilation (6). In the case of electrolytes composed of compound ions, like phosphates, with low ionization coefficients, the absorption of energy equivalent to the decomposition or discharge potentials greatly facilitates their assimilation. Maughan (19) found that radiation of 2,968 Ångstrom units or 4.16 equivalent volts is near the center of the most important radiation involved in the cure of rickets. This value is very near the decomposition or discharge potential of calcium phosphate, which is 4.20 equivalent volts. This shows that calcium phosphate is light stable to near

the short wave limit of the solar spectrum, which is around 4.26 equivalent volts (6). Either plants or animals using calcium phosphate will require light of high quality for its optimum assimilation. Ammonium and hydrogen phosphates, which have lower discharge potentials than calcium phosphate probably do not require as short waves of light for their assimilation. Therefore, plants which endure acid soil and relatively weak nutrient ions are often very tolerant of shade. As previously noted, phosphorous in the form of potassium phosphate is usually relatively easily assimilated because it has a high ionization coefficient, which probably facilitates the reduction of the phosphate ion to a phosphite ion. This reduction is probably necessary for the optimum assimilation of phosphorous. Since there is a positive correlation between the K_2O and P_2O_5 in the ash of pasture grasses and a negative correlation between the CaO and P_2O_5 in the ash content of pasture grasses, this suggests that potassium is often a very important element in the growth of early season plants such as Kentucky bluegrass. Available potassium can be increased by a direct application of potassium or indirectly by treating the soil with other material which may affect the availability of potassium in the soil.

Vitamins A and D present in cod liver oil probably have their origin in sea plants. Sea water might be considered as a rich nutrient medium, and as plants selectively absorb strong ions sea plants would probably require relatively short waves of light for their optimum development. The absorption of large quanta of energy would probably result in the synthesis of organic compounds which on oxidation would be capable of supplying large quanta of energy (6). Since life is a reduced system, the oxidation of organic materials will result in a free energy decrease and the emission of radiant energy. The size of the quanta emitted will probably be influenced by the energy level at which the various organic compounds are synthesized.

Von Euler, et al. (25) have identified vitamin A with lipochrome pigments. Positive results were obtained in growth experiments with carefully purified carotin from carrots as the sole source of vitamin A. They conclude that the effect of vitamin A in the blood is due to its oxidation-reduction action. Experiments with carotin by Moore (21) have confirmed some of the conclusions of Von Euler, et al.

Certain data (6) definitely suggest that many vital processes are on an energy level in close agreement with the free energy decrease in the formation of certain simple electrolytes, and with the reduction potentials of some common nutrient ions such as nitrates, borates, sulfates carbonates, and phosphates.

SUMMARY AND CONCLUSIONS

Data are reported on certain ash constituents of the grasses representing the most common plant successions accompanying the depletion of soils in New York pastures.

The nitrogen and the silica-free ash contents of the grasses seem to decrease as the soils become depleted.

Elements with relatively high standard electrode potentials, such as potassium and calcium, are often the predominant mineral constituents in the ash of plants.

A strong negative correlation was observed between the potassium and the calcium content of the ash of pasture grasses.

It is suggested that there is a correlation between the qualitative order of removal of atomic metallic cations from soil colloidal complexes by electro-dialysis and the order of the energy of removal, in equivalent volts, of the inmost normal valence electron of elements.

Many organisms differentially absorb atomic nutrient cations in the same qualitative order as they are removed from soil colloidal complexes by electro-dialysis.

Plants which require fertile soils or strong nutrient ions for optimum growth are often intolerant of shade and require large quanta of radiant energy for optimum growth. Plants which normally require a rich nutrient medium and large quanta of radiant energy may synthesize organic compounds which, on oxidation, would be capable of supplying large quanta of radiant energy and may be of high food value, whereas plants which grow normally on poor soils and endure weak nutrient ions are often shade tolerant and of relatively low food value.

The absorption of radiant energy of quality equivalent to or greater than the free energy decrease in the formation of simple electrolytes from the elements may greatly facilitate their assimilation by organisms.

There is a wide difference in the quality of radiant energy necessary for the reduction of the common nutrient anions. Phosphorous is one of the most difficult to reduce, ultraviolet light being required for its reduction. Phosphorous in compounds with relatively high ionization constants is often relatively easily assimilated, since phosphorous in ionic form is probably more easily reduced than is phosphorous in molecular form.

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INFLUENCE OF INORGANIC NITROGEN COMPOUNDS ON REACTION AND REPLACEABLE BASES OF NORFOLK SAND

R. M. BARNETTE AND J. B. HESTER

Florida Agricultural Experiment Station¹

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A number of grass plats have been established² at the Florida Agricultural Experiment Station at Gainesville for the purpose of studying the growth habits and the fertilizer requirements of lawn and golf green grasses in Florida. The plats are established for the most part on the typical deep phase of the Norfolk sand of this section. Clay lies from 8 to 10 feet below the substrata of yellow and surface soil of gray sand.

A series of these plats is devoted to lawn grasses, chief among which are Saint Augustine grass (*Stenotaphrum secundatum* Walt. Ktze.) and centipede grass (*Eremochloa ophiuroides* Munro. Hack). These plats were established in April, 1923, and are continued to date. One-third of each plat of grass is fertilized with sodium nitrate at the rate of 1,333 pounds an acre yearly applied in 12 monthly top-dressings. One-third receives ammonium sulfate at the rate of 1,000 pounds an acre yearly applied at the same time as the sodium nitrate and equivalent in nitrogen content to it. One-third remains unfertilized. During 1926 and 1927 four of these plats were selected for a study the effect of sodium nitrate and ammonium sulfate on the pH values of aqueous suspensions of the soil. Samples were made from the third of each of the four plats receiving the different nitrogenous fertilizer salts.

The determination of the H-ion concentration was made on the fresh samples, one part of the soil being used to approximately three parts of distilled water. The suspensions were allowed to stand overnight and the pH value of the clear supernatant liquid was determined by the indicator method with the double-wedge standards. The results were checked by two indicators when the range permitted.

The average results of 11 determinations on composite samples removed from the 0-6-inch depth of the soil from the variously fertilized areas of the plats during 1926 and 1927 are given in table 1.

It is noted that the individual parts of the plats receiving the same source of

¹ Contribution from the department of chemistry of the Agricultural Experiment Station, University of Florida, Gainesville, Florida.

² By the agronomy department of the Florida Agricultural Experiment Station in coöperation with the Bureau of Plant Industry, the U. S. Department of Agriculture and the U. S. Golf Association.

nitrogen vary very little within themselves. The results indicate that the sodium nitrate has increased the alkalinity of the soil and the ammonium sulfate has increased the acidity. The effect has not been great in either the acid or alkaline direction but the differences are significant because of the large number of determinations averaged, and the consistency of the results.

A series of plats devoted to the study of putting green grasses was sodded to various strains of Bermuda grass (*Capriola dactylon* L. Kuntze) in the spring of

TABLE 1

Average pH values of aqueous soil suspensions made on composite soil samples during 1926 and 1927*

TREATMENT	PLAT 1	PLAT 2	PLAT 3	PLAT 6	AVERAGE
	pH	pH	pH	pH	pH
No fertilizer.....	6.91	6.88	6.87	6.99	6.91
Sodium nitrate.....	6.98	7.00	6.98	7.02	7.00
Ammonium sulfate.....	6.63	6.72	6.82	6.70	6.71

* Average of 11 determinations at different times.

TABLE 2

Average pH values of aqueous soil suspensions made on composite soil samples during 1926 and 1927*

TREATMENT	PLAT 1	PLAT 2	PLAT 3	PLAT 4	PLAT 6	AVERAGE
	pH	pH	pH	pH	pH	pH

Norfolk sand

No fertilizer.....	6.54	6.55	6.59	6.61	6.61	6.58
Ammonium sulfate.....	6.26	6.27	6.19	6.24	6.41	6.15
Ammonium phosphate.....	6.40	6.31	6.35	6.41	6.44	6.38

Norfolk sand top-dressed with 2-3 inches of clay

No fertilizer.....	6.00	6.23	6.40	6.43	6.34	6.25
Ammonium sulfate.....	5.72	6.18	6.14	6.33	6.22	6.05
Ammonium phosphate.....	6.03	6.22	6.25	6.33	6.25	6.19

* Average of 11 determinations at different times.

1925. One half of each 10 by 25-foot plat received a top-dressing of approximately three inches of clay, the other half remaining the deep sandy soil. Across both the sand and clay parts of the plats, one-third of each plat is fertilized with ammonium sulfate and one-third receives ammonium phosphate (Ammono-phos, 20-20), whereas the middle third has no fertilizer treatment. The ammonium sulfate and ammonium phosphate are applied at the rate of 1,000 pounds an acre yearly in fractional semi-monthly top-dressings. Five of these plats were selected for a study of the influence of ammonium sulfate

and ammonium phosphate on the pH values of aqueous suspensions of the soil. Samples were taken from both the clay top-dressed and sandy ends of the plats to a depth of approximately six inches. Eleven determinations of the pH values of the aqueous suspensions were made during 1926 and 1927 by the method described. The averaged figures obtained on the various plats are given in table 2.

The ammonium sulfate has lowered the pH value of an aqueous suspension of the soil more than has the ammonium phosphate. The change has been greater in the sand than in the clay top-dressed halves of the plats. These results are in accord with the data of Pierre (2) who found that ammonium sulfate increased the H-ion concentration of aqueous soil suspensions more than did ammonium phosphate and that the change in H-ion concentration induced by a given nitrogenous source was dependent upon the buffer capacity of the soil.

TABLE 3
Effect of increasing amounts of ammonium sulfate on the pH value

YEARLY ADDITIONS OF AMMONIUM SULFATE TO THE ACRE	ORIGINAL	3 MONTHS*	8 MONTHS†	10 MONTHS‡	21 MONTHS
pounds	pH	pH	pH	pH	pH
None	6.76	6.63	6.53	6.33	6.47
1,000	6.78	6.51	6.44	6.29	6.21
2,000	6.73	6.39	6.31	6.12	5.89
3,000	6.45	6.37	6.30	6.05	5.43
5,000	6.60	6.30	6.02	5.89	4.45

* Average of determinations made at end of second and third month after first application of ammonium sulfate.

† Average of determinations made at end of fourth, fifth and sixth month.

‡ Average of determinations made at end of ninth and tenth month.

A third series of plats was established to Bermuda grass in 1926 and increasing quantities of ammonium sulfate, applied every two weeks as a dressing, are used as a source of nitrogen on these plats. A series of samples representing a composite sample of the 0-12-inch depth of the soil of these plats was taken for about 21 months after the beginning of the experiment.

The pH values of the variously fertilized soils are given in table 3 for the different dates after the initiation of the experiment. In 21 months, the higher applications of ammonium sulphate have decreased the pH values of the aqueous soil suspensions from the original pH of approximately 6.6 to as low as pH 4.45, in the case of the 5000-pound application. This indicates the low buffer capacity of the deep phase of the Norfolk sand in Florida. The increase in H-ion concentrations has been proportional to the application of ammonium sulfate.

It is generally conceded that the removal of the bases of the soil by leaching

TABLE 4
Effect of increasing amounts of ammonium sulfate on replaceable bases and on the pH value of aqueous suspensions at different depths
 Per 100 gm. of air-dried soil

ADDITIONS* OF AMMONIUM SULFATE	0-9 INCHES						9-21 INCHES						21-33 INCHES					
	Ca	Mg	Na	K	Total	pH	Ca	Mg	Na	K	Total	pH	Ca	Mg	Na	K	Total	pH
	m.e.	m.e.	m.e.	m.e.	m.e.		m.e.	m.e.	m.e.	m.e.	m.e.		m.e.	m.e.	m.e.	m.e.	m.e.	
None	2.464	0.800	0.194	0.149	3.607	6.57	1.964	0.500	0.129	0.085	2.678	6.66	1.821	0.400	0.194	0.149	2.564	6.28
1,000	1.929	0.650	0.226	0.128	2.933	6.16	1.786	0.600	0.161	0.149	2.696	6.26	1.750	0.350	0.129	0.106	2.335	6.05
2,000	1.179	0.550	0.194	0.149	2.072	6.01	1.321	0.500	0.194	0.085	2.100	6.18	1.286	0.400	0.097	0.085	1.868	5.84
3,000	0.928	0.400	0.161	0.085	1.574	5.76	1.393	0.350	0.065	0.085	1.893	5.93	1.557	0.400	0.097	0.064	1.918	5.76
5,000	0.786	0.400	0.065	0.106	1.357	5.59	1.179	0.300	0.065	0.064	1.608	5.76	1.250	0.350	0.097	0.064	1.761	5.76

* Additions on basis of pounds an acre yearly.

and by plants and the replacement of the cations Ca, Mg, Na, and K by the H ion is responsible for the development of an acid condition in the soil. In order to study the change in the replaceable base content of the deep phase of the Norfolk sand as induced by the different applications of ammonium sulfate, a series of samples at different depths was taken on August 4, 1927, or 17 months after the initial applications of ammonium sulfate and the establishment of the plats to Bermuda grass.

Six samples were taken from each plot to make a composite sample, each of which was thoroughly mixed and air-dried. Fifty grams of the samples were leached with one liter of $N\ NH_4Cl$ to determine the replaceable cations. It was found necessary to use these proportions to obtain quantities of the bases which could be estimated with a fair degree of accuracy. For the determination of the Ca and Mg, 300 cc. of the ammonium chloride leachings were used, and 600 cc. were used for the estimation of the Na and K. The pH values were determined in a 1-2 soil-water suspension by the quinhydrone method.

TABLE 5

Effect of increasing amount of ammonium sulfate on relative total m.e. of replaceable bases

Untreated soil taken as basis

ADDITIONS OF* AMMONIUM SULFATE	0-9 INCHES	9-21 INCHES	21-33 INCHES
<i>pounds</i>			
None	100.0	100.0	100.0
1,000	81.3	100.6	91.0
2,000	57.5	78.5	72.8
3,000	43.6	70.6	74.8
5,000	37.6	60.0	68.6

* Additions on basis of pounds an acre yearly.

The replaceable cation content expressed as milligrams equivalent (m.e.) per 100 gm. of air-dried soil and the pH values of the aqueous soil suspensions are given in table 4. Increasing amounts of ammonium sulfate decrease the replaceable cation content of the soil. There is a correlation in the pH value of the aqueous suspension of the soil and the replaceable cation content for each depth of the soil. The higher the replaceable cation content of a given depth of soil the higher the pH value. The total capacity of the soil to combine with bases evidently decreases with depth, the higher content of the replaceable cations in the surface soil being associated with a higher organic matter content.

The replaceable cation content of the untreated soil being considered as 100, the percentages of the total replaceable cations found in the ammonium sulfate treated plats are calculated and given in table 5. From these calculations it can be seen that there is a decided decrease in the removal of the replaceable bases with an increase in the depth of the soil. There is also a corresponding

decrease in the effect of the ammonium sulfate in lowering the pH values of the lower depths of the soil. This is in accord with the results of Crowther (1) who found that there was a depth distribution of the effect of ammonium sulfate in decreasing the pH value of soils at Woburn and Rothamsted.

The divalent cations predominate in the soil complex. Calcium is presenting amounts far in excess of the other cations. In table 6 are given the relative proportions of the different cations in the replaceable state in the soil and the effect of the ammonium sulfate in changing these proportions in the various

TABLE 6

Distribution of Ca, Mg, Na, and K in every 100 m.e. of the replaceable cations in three depths of soil

ADDITIONS OF* AMMONIUM SULFATE	0-9 INCHES				9-21 INCHES				21-33 INCHES			
	Ca	Mg	Na	K	Ca	Mg	Na	K	Ca	Mg	Na	K
<i>pounds</i>												
None	68	22	5	4	73	19	5	3	71	16	8	6
1,000	66	22	8	4	66	22	6	6	75	15	6	5
2,000	57	27	8	7	63	24	9	4	69	21	5	5
3,000	59	25	10	5	74	19	3	5	71	21	5	3
5,000	58	30	5	8	73	19	4	4	71	20	6	4

* Additions on basis of pounds an acre yearly.

TABLE 7

Percentages of the total m.e. of cations lost by the ammonium sulfate treated plats at different depths accounted for by divalent and monovalent cations†

ADDITIONS OF* AMMONIUM SULFATE	0-9 INCHES		9-21 INCHES		21-33 INCHES	
	Ca + Mg	Na + K	Ca + Mg	Na + K	Ca + Mg	Na + K
<i>pounds</i>						
1,000	100.00	100.00	52.84	47.16
2,000	100.00	100.00	76.87	23.13
3,000	95.23	4.77	91.84	8.16	71.83	28.17
5,000	92.36	7.64	92.06	7.94	77.33	22.67

* Additions on basis of pounds an acre yearly.

† Calculations made on the basis of replaceable base content of the untreated plat.

depths. In these calculations the H ion and the Al ion have not been taken into consideration. As can be seen, the calcium is removed to a greater extent than the other cations.

With the cation content of the untreated soil as a basis, the losses in cations as distributed between the divalent and the monovalent ions have been calculated and the percentages of distribution are given in table 7. Although in all depths of the soil the divalent Ca and Mg make up the greater part of the loss, still in the 21-33-inch depth, particularly, the monovalent Na and K

account for a very much larger proportion of the loss, indicating a replacement of these cations by the Ca and Mg.

SUMMARY

A study was made of the influence of different carriers of nitrogen on the H-ion concentration of the deep phase of a Norfolk sand which was sodded to lawn and golf green grasses. The influence of increasing applications of ammonium sulfate on the replaceable cation content of the deep phase of the Norfolk sandy soil was also studied for the different depths of the soil. The results may be summarized as follows:

Ammonium sulfate increased the acidity and sodium nitrate the alkalinity of the deep phase of the Norfolk sand sodded to lawn grasses.

Ammonium sulfate increased the acidity of the deep phase of the Norfolk sand more than did ammonium phosphate which had been applied in amounts equivalent in nitrogen content.

A dressing of three inches of clay decreased the effect of both ammonium sulfate and ammonium phosphate in lowering the pH value of Norfolk sand (deep phase).

The decrease in H-ion concentration of the Norfolk sand (deep phase) induced by applications of ammonium sulfate was proportional to the amounts of ammonium sulfate applied, and correlated with a decrease in the total milligram equivalents cations in the replaceable state in the soil.

The effect of ammonium sulfate on the removal of replaceable cations and the effect on the H-ion concentrations of the soil decrease with depth of soil.

The divalent cations are predominate in the Norfolk sand (deep phase).

The application of ammonium sulfate decreases the replaceable divalent cation content to a greater extent than the replaceable monovalent cation content.

The losses of the replaceable cations in the Norfolk sand (deep phase) indicate a replacement of the monovalent cations in the lower depths of soil by the divalent cations.

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NITROGEN AND ORGANIC MATTER IN HAWAIIAN PINEAPPLE SOILS

A. L. DEAN

Experiment Station, Association of Hawaiian Pineapple Cannors

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The following data are presented because of their possible bearing on the commonly accepted view that the carbon and nitrogen of soils tends toward a ratio of 10 to 1, and their failure to harmonize with the results reported by Jenny (1, 2) in his study of the influence of climate on soil nitrogen. Jenny does not attempt to extend the validity of his conclusions beyond the continental portions of the United States. His data are derived from the analyses of soils in central United States, chiefly in the regions between the Mississippi River and the Rocky Mountains, and support his conclusion that the nitrogen of the soil decreases with increase of the mean annual temperature.

The 223 soil samples, the nitrogen and organic content of which are here summarized, were taken from pineapple fields, or lands destined to become pineapple fields. They came from the islands of Hawaii, Maui, Lanai, Molokai, Oahu, and Kauai, from elevations ranging from 200 feet to 3,000 feet above sea level, and from locations where the rainfall ranges from less than 20 inches to approximately 100 inches yearly. The great majority of the samples were taken from the surface to a depth of 12 inches, a few to 18 or 20 inches, and in some cases, where the topsoil was thin, the sampling did not extend below 8 or 10 inches. Some of the soils were from uncultivated ranch lands, some had been in pineapples for 20 years or more. In short, they are an unselected group of soils, representing many thousands of acres of unirrigated lands, from localities where they have been exposed to different conditions. They have a similar origin in that they are all derived from basaltic lavas or cinders. The mean annual temperatures have not been precisely the same for all of them, but a representative figure would be 70°F. They would probably all fall within the range of 65° to 75°F. This is comparable to the warmest regions included in Jenny's series. The Hawaiian climate, however, is less variable than that of Texas and Louisiana and temperatures more than 15°F. on either side of the mean are unusual.

The organic matter has been determined¹ by the method of J. B. Rather (4).

¹ None of the analyses were made by me and they were not carried out for the purpose of investigating the subject here discussed. Prof. F. T. Dillingham of the University of Hawaii, and Messrs. F. A. E. Abel, Carl A. Farden, and L. A. Dean have done the analytical work.

The procedure was as follows:

Reagents: Acid—washed and ignited asbestos, 2.5 per cent hydrochloric acid, 2.5 per cent hydrofluoric acid.

Procedure: Weight out, by difference, 1-gm. samples of oven-dry soil [prepared as in the Official Methods of the Association of Official Agricultural Chemists edition of Sept. 1920, page 309, 2 (a)] into platinum dishes. Measure into a glass cylinder 30 cc. distilled water, 10 cc. 2.5 per cent HCl, and 10 cc. 2.5 per cent HF; mix, and pour the mixture carefully upon the soil sample. Place the whole upon a boiling water bath and digest it for exactly five minutes.

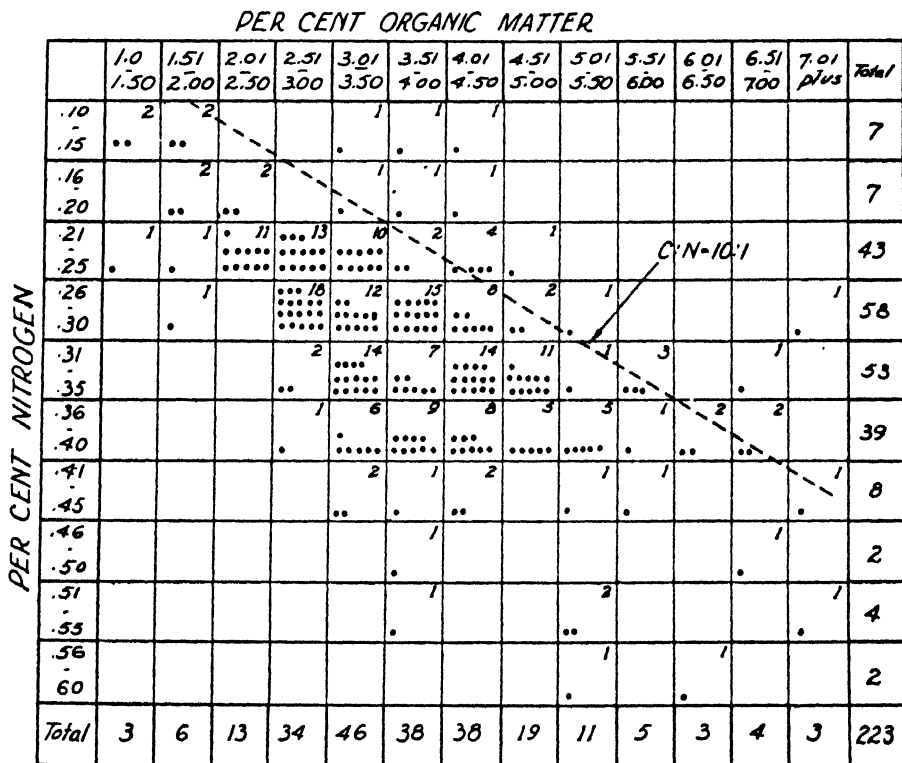


FIG. 1. DISTRIBUTION OF NITROGEN AND ORGANIC MATTER IN 223 HAWAIIAN SOILS

Remove it from the bath, allow it to settle for about three minutes, and decant it through a Gooch crucible containing a thin felt of washed and ignited asbestos, using suction.

To the residue in the dish add a second mixture of 30 cc. water, 10 cc. 2.5 per cent HCl and 10 cc. 2.5 per cent HF. Digest again on the water bath for exactly five minutes, allow to settle, and decant through the Gooch crucible.

Repeat the foregoing digestions and decantations four times more, making six separate digestions in all. Then transfer the soil residue completely to the crucible, using hot water and a policeman. Wash thoroughly with hot water, using suction.

Dry the crucible and contents over night in the electric oven at 95° to 100°C. Cool in a desiccator and weigh. Ignite the crucible and contents at low redness for one hour. Cool and weigh. The loss in weight resulting from ignition represents organic matter.

Too high results are probable, because of incomplete removal of the hydrated colloids of the soil. It appears unlikely that the percentages of organic matter reported are too low.

The accompanying distribution diagram summarizes the results. The mean nitrogen content is 0.31 ± 0.004 per cent, and that of the organic matter is 3.75 ± 0.03 per cent. The coefficient of correlation between nitrogen and organic matter is $+0.74$. Assuming a factor of 1.72 for carbon to organic matter, we get an average carbon percentage of 2.18 and a carbon-nitrogen ratio of 7 to 1. The 10 to 1 line has been drawn across the diagram and almost all of the samples are shown to be too rich in nitrogen to conform to that value.

There was a tendency for the very dry and very wet lands to run somewhat low in organic matter. Some of the driest districts have an annual rainfall well below 20 inches, which is rather dry in a subtropical region. In such places the organic matter is frequently less than 2 per cent. Some of the wettest districts have an excessive leaching of the soil, with resulting soils of low fertility and pH values around 4.0. The districts of moderate rainfall tend to have soils which are well provided with organic matter.

It is clear that the nitrogen content of our soils does not agree at all with the data presented by Jenny. To be consistent, this average should be less than 0.1 per cent, whereas it is three times that. On the basis of soil nitrogen, Hawaii belongs just south of the Canadian boundary instead of south of the Tropic of Cancer.

A number of years ago, Kelley (3) emphasized the inert character of the nitrogen in the uncultivated Hawaiian soils. He attributed this to lack of aeration. The cultivated soils of the pineapple industry receive an unusually thorough mechanical preparation; six to ten plowings extending over a period of one or two years are customary. Nitrification of added fertilizers takes place rapidly in these cultivated lands. Thoroughly prepared lands, before being fertilized and planted, sometimes show nitrate nitrogen contents between 50 and 100 p.p.m., and commonly over 20 p.p.m. A careful check on the organic matter of a number of virgin soils which were broken up for pineapples has shown a drop in this component with a tendency for the soils to become stable at some percentage between 3 and 4.

Whatever may be the reasons, it seems fairly evident that our Hawaiian soils are rich in nitrogen as compared with the southern states, and that their organic matter contains a higher percentage of nitrogen than the usual run of soils of the temperate zone. This latter observation is consistent with the tendency for the carbon-nitrogen ratio to become narrower with increasing mean annual temperature as brought out by Jenny (2).

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THE OXIDATION OF PYRITE AND SULFUR AS INFLUENCED BY LIME AND MAGNESIA—A 12-YEAR LYSIMETER STUDY

W. H. MacINTIRE, W. M. SHAW, AND J. B. YOUNG

The University of Tennessee Agricultural Experiment Station

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In previous lysimeter studies at this station it was found that different forms and amounts of lime and magnesia exerted divergent effects upon the leachings of sulfates that were derived from the soil and rainfall (4, 8, 9). In the early period of a subsequent experiment a similar divergence was obtained when sulfur additions were made in three forms (10). Further studies developed the reasons for the fixation of the sulfate radical by CaO (5, 6). The present contribution gives the results obtained in a 12-year lysimeter study of the oxidation of pyrite and elemental sulfur with ferrous sulfate control, as influenced by lime and magnesia. The changes that transpired in the soil are considered on the basis of: the recovery of sulfur as leached sulfate; the outgo of calcium, magnesium, and potassium; and the outgo of nitrates.

EXPERIMENTAL

The project was begun August 3, 1917 and terminated August 3, 1929. The average annual rainfall during that period was 51 inches. A soil of the Hagerstown series, a brown, fairly fertile loam of a slightly sandy phase, was used without subsoil. The soil had a total S content of 0.0326 per cent and gave an electrometrical pH value of 6.27 in a 1-10 suspension after one hour. As determined by Na_2O_2 - Na_2CO_3 fusion, the total CaO and MgO contents of the soil were 0.217 per cent and 0.398 per cent, or CaCO_3 -equivalences of 0.388 per cent and 0.995 per cent, respectively.

The sulfur additions—ferrous sulfate, pyrite, and elemental sulfur—were made at the constant rate of 1,000 pounds of S to 2,000,000 pounds of soil. The sulfur contents of the three materials were 18.15 per cent, 51.95 per cent, and 99.89 per cent, respectively. Pyrite was included, on the supposition that this cheap mineral would oxidize slowly and thus insure a uniform supply of sulfates. The ferrous sulfate was included to eliminate the effect of any additive base, to parallel the effect of iron in the pyrite, and to provide an absolute check on the outgo of the sulfate radical, instead of depending upon theoretical computations.

Each sulfur carrier was used alone and with lime and magnesia at the CaO-equivalent rates of 1 ton and 32 tons. The actual acidity of the ferrous sulfate, and that possible from the full oxidation of the pyrite and sulfur, was equivalent

to 1,750 pounds of CaO. The 1-ton lime and magnesia treatments were therefore supplemented by this amount. This supplement of CaO or MgO was not neutralized with uniform rapidity. The difference between the immediate action of the ferrous sulfate and the slower action of the generated sulfate radical was cared for by the use of both 2,000-pound and 3,750-pound controls. Limestone and dolomite of 100-mesh fineness, and at the CaO-equivalent rate of 2,000 pounds, or 3,570 pounds of CaCO_3 , were also included in the group of controls.

SULFATE OUTGO

The sulfur additions, increments, and recoveries are expressed throughout as pounds of S to 2,000,000 pounds of soil. The percolated sulfates are considered as a measure of the speed and ultimate extent of the oxidation of pyrite and elemental sulfur. No other record of the use of either sulfate or pyrite in lysimeter studies has been found. Recently, however, ferrous sulfate was used as a soil amendment by Kelley and Arny (3) in extensive laboratory-controlled plat studies with alkali soils, and the oxidation of heavy pyrite additions in soil cultures was studied by Rudolfs (11).

No-sulfur-addition group. The data of the first group in table 1 show that all of the six liming treatments produced an increase in the outgo of sulfates. In general, the increases were accounted for during the first two years. The effects of the four 2,000-pound additions were comparable. The effects of the 1,750-pound supplements of CaO and MgO were definite, though not extensive. It therefore appears that the variations in the rapidity with which the 1,750-pound supplements were neutralized did not constitute an important factor in the sulfate outgo from the soil itself.

The relation between increment of sulfates from rainwaters and the outgo of sulfates from the no-sulfur control group is shown in table 2. There was no actual loss of sulfates from the untreated soil through leaching, since the outgo from the control was only 78.8 per cent of that brought down by rain. This relationship between increment and outgo was also found recently for a similar soil by Ellett and Hill (2). Neither of the limestones, nor the equivalent oxide treatments, produced a sulfate leaching equal to that derived from the rain, 514 pounds. The respective losses from the 3,750-pound lime and magnesia additions exceeded the sulfates derived from rainwater by only two per cent and four per cent. Increases in outgo of sulfates, as a result of liming, were also found by Ellett and Hill (2) for those soils that were not underlaid by subsoil. Economic liming might be considered as causing a decrease in the capacity of the soil to retain sulfates. In view of the immediate enhancement in the sulfate content of the percolates, however, it is more probable that oxidation of the supply of organic sulfur in the soil was stimulated by the liming.

Ferrous sulfate group. The ferrous sulfate control gave a large sulfate outgo during the first year. This outgo was, however, only a fraction of the greater losses induced by the 1-ton supplements of the two oxides, and only 53 per cent

of the outgo from the heavy MgO addition. The 32-ton addition of burnt lime resulted in the formation of the compound $3 \text{CaO} \cdot \text{Al}_2\text{O}_3 \cdot 3 \text{CaSO}_4 \cdot 33 \text{H}_2\text{O}$, which is insoluble during the persistence of $\text{Ca}(\text{OH})_2$; hence the sulfate outgo from the heavily limed ferrous sulfate addition was greatly retarded (6).

TABLE 1

The 12-year outgo of sulfate sulfur derived from 1,000-pound S equivalence additions of ferrous sulphate, pyrite and sulfur to a loam soil, as influenced by lime and magnesia supplements

TANK NUMBER	LIME OR MAGNESIA ADDITIONS		SULFUR ADDITION	ANNUAL OUTGO OF S IN POUNDS FROM 2,000,000 POUNDS OF SOIL												
	Form	Rate*		First	Second	Third	Fourth	Fifth	Sixth	Seventh	Eighth	Ninth	Tenth	Eleventh	Twelfth	Total 12 years
50	None	None	18	37	47	41	34	32	18	19	37	41	37	44	405
51	L.S.	2,000	None	58	55	44	41	35	30	23	24	41	40	42	39	472
52	Dol.	2,000	None	62	56	46	41	37	29	24	30	48	46	46	42	507
53	CaO	2,000	None	62	59	48	45	34	32	23	27	43	44	43	43	503
54	CaO	3,750	None	81	56	49	40	35	30	26	30	46	42	43	47	525
55	MgO	2,000	None	63	53	47	43	35	33	25	28	40	44	42	41	494
56	MgO	3,750	None	94	57	51	43	34	31	26	30	40	41	45	44	536
57	None	FeSO ₄	534	180	109	68	42	44	23	26	37	55	40	50	1,208
58	CaO	3,750	FeSO ₄	829	173	74	50	38	35	24	27	45	47	42	43	1,427
59	MgO	3,750	FeSO ₄	940	129	61	46	37	34	26	31	42	47	47	44	1,484
60	CaO	32T	FeSO ₄	82	178	264	221	147	39	31	33	49	45	49	47	1,185
61	MgO	32T	FeSO ₄	1,007	184	55	41	34	34	23	27	45	49	39	48	1,586
62	None	Pyrite	183	279	206	116	87	62	30	34	52	66	43	57	1,215
63	CaO	3,750	Pyrite	189	363	154	120	92	75	51	53	67	66	58	56	1,344
64	MgO	3,750	Pyrite	184	255	133	84	56	48	38	42	57	56	59	52	1,064
65	CaO	32T	Pyrite	32	67	97	132	87	130	114	161	111	94	85	77	1,187
66	MgO	32T	Pyrite	166	107	79	54	50	65	40	42	59	61	52	68	843
67	None	Sulfur	507	211	118	70	50	43	25	25	46	55	42	50	1,242
68	CaO	3,750	Sulfur	734	236	78	51	38	31	23	29	42	42	43	42	1,389
69	MgO	3,750	Sulfur	817	219	70	50	37	34	26	29	40	46	48	43	1,459
70	CaO	32T	Sulfur	89	205	263	218	117	47	35	34	48	47	52	41	1,196
71	MgO	32T	Sulfur	426	608	117	51	37	35	22	26	48	47	39	47	1,503
Rainfall.....inches				41	51	55	56	51	55	44	35	51	59	55	58	514
Average.....																51

* Pounds, or tons, CaO \approx to 2,000,000 pounds of soil, moisture-free basis.

The sulfate outgo from the ferrous sulfate control decreased decidedly after the first year; the difference between the loss from it and the loss from the untreated soil at the end of the third year was 721 pounds, as compared with 803 pounds after 12 years. Thus, in spite of 12 years of leaching, there was a re-

tention of 20 per cent of the sulfur added in the ferrous sulfate control. This indicates that insoluble sulfate compounds were formed in the soil. On the other hand, it is possible that there was a loss of sulfur as a result of reduction in the acid soil. No ferrous sulfate appeared in the early leachings from any of the ferrous sulfate tanks. The increase in the sulfate radical was accounted for by enhancements in leachings of calcium and magnesium. This does not confirm the viewpoint of Van Bemmelen (12) that the sulfuric acid radical combines with iron in the soil to protect the alkaline-earth bases against solvent action and leaching. It does accord, however, with the conclusions of Kelley and Arny (3) relative to the reactions that ensue between ferrous sulfate and exchangeable calcium and magnesium.

When the sulfate losses from the two light additions of oxides and the heavy addition of magnesia are corrected for outgo from the untreated soil, the recoveries are more than equal to the sulfate content of the ferrous sulfate addition.

Table 1 shows the more rapid outgo of magnesium sulfate. After the third year, however, the outgo for the two MgO additions and the 1-ton CaO addition was almost identical. The initial sulfate fixation by the heavy lime treatment was not permanent and the outgo for sulfate alone and for sulfate plus 32 tons of lime, was practically identical during the last seven years. The common-ion effect is not a potent factor during the period subsequent to the conversion of the hydrated lime into calcium carbonate.

Sulfur group. Because of its similarity to the sulfate group, and its dissimilarity to the pyrite series, the elemental sulfur group will next be considered. At the end of the first year the sulfate outgo from the unlimed sulfur addition was only 11 pounds less than one half the amount added. The outgo from the sulfur control was practically the same as that from the ferrous sulfate control by periods and for the 12-year total. Even without liming, the oxidation of the sulfur kept pace with the removal through leaching. The 1-ton additions of lime and magnesia caused distinct acceleration in sulfur oxidation and gave net sulfate recoveries of 91.5 per cent and 98.1 per cent, respectively, during the first two years. The heavy lime addition caused the same marked decrease in sulfate outgo that was found for ferrous sulfate. It is most probable that the intense alkalinity produced by the heavy addition of lime was inhibitive to the biological oxidation of elemental sulfur. Nevertheless, it is not proved that the heavy lime additions did produce this effect, for had sulfates been present as they were in the ferrous sulfate series, they would not have come out in the percolates during the persistence of the $\text{Ca}(\text{OH})_2$. The explanation for this chemical phenomenon has been given in the discussion of the ferrous sulfate data.

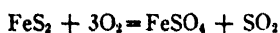
The first annual outgo of sulfates from the sulfur addition with 32 tons of magnesia amounted to only 84 per cent of that obtained from the sulfur alone, and only 42.3 per cent of the 1,007-pound outgo from the corresponding ferrous sulfate addition. At the end of two years, however, the heavy magnesia

treatment gave a net sulfate recovery of 97.9 per cent of the sulfur addition. Hence, though delayed in starting, the sulfate recovery from the heavier magnesia addition caught up with that from each oxide at the lighter rate, and ultimately was the largest of the sulfur group. Data in table 1 show that, at the 1-ton rate, both oxides gave a full sulfur recovery, but there was a disparity of 31.2 per cent between the excess recovery from 32 tons of magnesia and the minus value found for the corresponding addition of burnt lime.

Since the same heavy sulfate outgo was produced from this soil by the 32-ton additions without any added sulfur (8, 9), it would appear that the oxidation of elemental and native organic sulfur was induced by the same, or a similar, organism.

Pyrite group. In this group the oxidation process was immediate and positive, save for the 32-ton addition of burnt lime. Nevertheless, the respective generations of sulfates were not nearly so rapid as in the sulfur group. In table 1 it is shown that no pyrite addition gave a 100 per cent recovery, and only in the case of the 1-ton CaO treatment was the enhanced outgo greater than that from the unsupplemented pyrite. The descending order in sulfate outgo from heavy magnesia, light magnesia, light lime, control, and heavy lime that was found for both the ferrous sulfate and elemental sulfur, was changed materially in the pyrite group. The order of outgo from the first four years was, pyrite plus one ton of CaO, pyrite alone, and pyrite plus one ton of MgO, with respective losses of 826, 784, and 656 pounds. This order did not hold, however, for the full 12-year period. The greatest total loss—1,344 pounds for the 1-ton CaO addition—was followed by an outgo of 1,215 pounds from the pyrite alone, but the ultimate loss induced by 32 tons of CaO was greater than the outgo caused by the light addition of magnesia. In decided contrast to the ferrous sulfate and elemental sulfur groups, the minimum loss of sulfates came from the 32-ton addition of magnesia in spite of the initial lag in the outgo from the heavy lime addition. The minimum sulfate outgo from the pyrite and heavy magnesium oxide cannot be explained by assuming that *magnesium sulfite* was formed through reaction between SO₂ and hydrated magnesium oxide, for this salt is readily soluble in water, and if leached it would have been converted to magnesium sulfate during the analytical procedure. The data of the ferrous sulfate and elemental sulfur groups show that magnesium sulfate would have come out, had it been present. Hence, it is evident that the heavy magnesia additions had a depressive effect upon the production of sulfates from the pyrite. If it be assumed that the pyrite was oxidized mainly through bacterial action, it follows that the organism that effected oxidation of the pyrite was different from that which caused oxidation of the elemental sulfur.

The oxidation of pyrite may be effected in part by bacteria, as was concluded by Rudolfs (11); nevertheless, the mineral sulfide readily undergoes a purely chemical change into ferrous sulfate, as indicated by the equation,



Distinct evidence of SO_2 is obtained from samples of the finely ground material that have been stored in corked bottles. We have confirmed the observations of Allen and Johnston (1) to the effect that considerable quantities of ferrous sulfate are generated when pyrite is ground to obtain an analytical charge. It is quite probable that the generation of sulfates from the added pyrite was therefore due in considerable part to purely chemical action.

The rates of cumulative outgo of sulfate-sulfur from 1,000-pound S-equivalent additions of pyrite with and without lime and magnesia (table 1) are materially different from those resulting from the addition of elemental sulfur and ferrous sulfate. The total recovery of 1,215 pounds from the pyrite control is in remarkable concordance with the 1,208-pound and 1,242-pound recoveries from the ferrous sulfate, and sulfur controls, respectively. A similar concordance was found in the yields of 1,185, 1,187, and 1,196 pounds from the same three materials with the 32-ton CaO supplement. Only in the case

TABLE 2
Total sulfate S outgo from soil without sulfur increment except through rainfall

SULFATE S PER 2,000,000 POUNDS OF SOIL, MOISTURE-FREE BASIS	IN PERCOLATES FROM SOIL WITH ADDITIONS OF						
	No treat- ment	100-mesh, 2,000- pounds CaO - Equivalent		Burnt lime		Burnt magnesia	
		Lime- stone	Dolo- mite	2,000-lb rate	3,750-lb rate	2,000-lb ∞	3,750-lb. ∞
Actual outgo.....pounds	405	472	507	503	525	494	536
Increase over control.....pounds	67	102	98	120	89	131
Increase over control.....per cent	16.5	25.2	24.2	29.6	22.0	32.3
Outgo, as per cent of rainfall increment..	78.8	91.8	98.6	97.9	102.1	96.1	104.3

of the pyrite plus 32 tons of CaO , with its early inhibitive action, is there to be found an outgo of sulfates that is not in harmony with the recoveries from the other treatments during the last 5-year period, and it is evident that a state of virtual equilibrium between sulfur supply and sulfate outgo has been reached.

OUTGO OF CALCIUM, MAGNESIUM, AND POTASSIUM

Calcium-magnesium outgo

Each calcium and magnesium outgo is expressed as pounds CaCO_3 -equivalent for each 2,000,000 pounds of soil.

Control group. The data of table 3 show practically the same outgo of calcium for the 2,000-pound equivalences of high-calcic lime and limestone. A larger outgo came from the 3,750-pound CaO addition. This larger outgo was evident in each of the 12 annual periods and aggregated 1,227 pounds of CaCO_3 -equivalence. By an increase of seven-eighths of the lime added, the enhanced outgo was 2.66 times the actual loss from the 1-ton CaO addition. Each increment of magnesium depressed the outgo of calcium, and the 3,750-

pound MgO addition was more repressive than the 2,000-pound treatment. This repressive effect was exerted by the magnesium content of the dolomite, as well as the oxides of magnesium, as has been noted and explained in a previous contribution (7).

The magnesium leachings from the high-calcic additions were uniformly lower than the magnesium outgo from the untreated soil (fig. 1). The dolomite gave a magnesium outgo practically double that from the untreated soil.

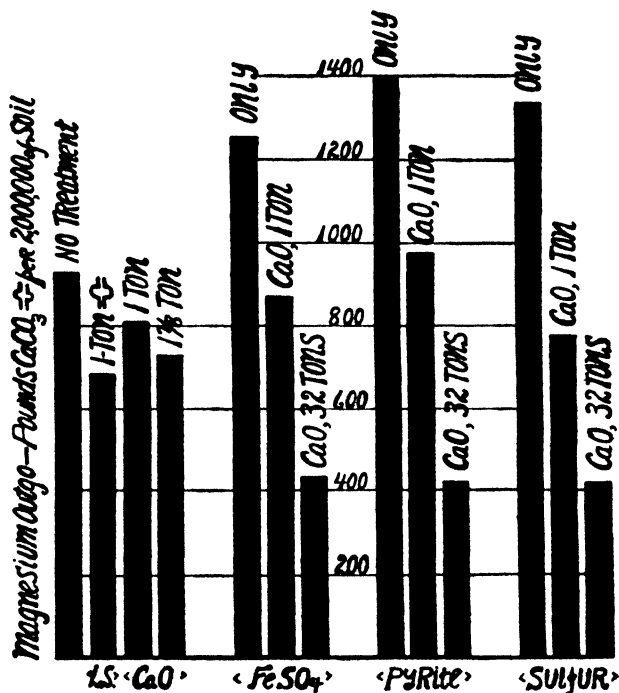


FIG. 1. THE REPRESSIVE EFFECT OF UNSUPPLEMENTED ADDITIONS OF LIMESTONE AND BURNT LIME UPON THE OUTGO OF NATIVE SOIL MAGNESIUM; THE ENHANCEMENT IN OUTGO OF NATIVE MAGNESIUM INDUCED BY UNSUPPLEMENTED ADDITIONS OF FERROUS SULFATE, PYRITE, AND ELEMENTAL SULFUR; AND THE DEPRESSED OUTGO OF NATIVE MAGNESIUM FROM FERROUS SULFATE, PYRITE, AND ELEMENTAL SULFUR WHEN SUPPLEMENTED WITH BURNT LIME AT TWO RATES

Since the 100-mesh dolomite addition had been absorbed before the end of the first year, this enhancement is to be attributed to the greater hydrolytic disintegration of the magnesium absorption complex. The complete removal of the absorbed magnesium would account for about 87 per cent of the magnesium outgo. The enhancement in the magnesium outgo from the 3,750-pound MgO addition was exactly 1.875 times that from the 1-ton addition.

The persistence of liming materials in the soil is considered in the summaries given in table 4. The total calcium plus magnesium outgo from the 2,000-pound CaO-equivalences of burnt lime, limestone, and dolomite did not differ materially, although that from the dolomite was the greatest. The maximum

TABLE 3

The 12-year outgo of calcium from lime and magnesia additions to a loam soil, as influenced by 1,000-pound sulfur additions in the forms of ferrous sulfate, pyrite, and pulverized sulfur

TANK NUMBER	LIME OR MAGNESIA ADDITIONS		SULFUR ADDITIONS	ANNUAL OUTGO OF CALCIUM AS CaCO ₃ ≈ POUNDS TO 2,000,000 POUNDS OF SOIL												
	Form	Rate*		First	Second	Third	Fourth	Fifth	Sixth	Seventh	Eighth	Ninth	Tenth	Eleventh	Twelfth	Total 12 years
50	None	None	292	248	234	260	188	143	108	89	223	174	195	171	2,325
51	L.S.	2,000	None	370	406	336	351	201	156	148	128	282	204	235	209	3,026
52	Dol.	2,000	None	230	273	227	219	166	132	87	83	213	171	177	122	2,100
53	CaO	2,000	None	406	408	278	371	226	179	150	149	289	224	231	167	3,078
54	CaO	3,750	None	697	587	477	483	293	234	170	167	366	274	291	266	4,305
55	MgO	2,000	None	155	147	166	161	111	98	50	61	128	100	125	98	1,400
56	MgO	3,750	None	155	143	166	160	96	67	42	42	108	77	90	102	1,248
57	None	FeSO ₄	1,348	611	374	278	172	116	76	87	169	146	137	132	3,646
58	CaO	3,750	FeSO ₄	2,607	718	351	338	205	179	151	155	311	237	229	220	5,701
59	MgO	3,750	FeSO ₄	582	139	121	132	89	46	45	48	101	83	88	76	1,550
60	CaO	32T	FeSO ₄	3,801	1,653	1,863	1,350	1,229	833	500	552	818	820	882	981	15,282
61	MgO	32T	FeSO ₄	79	60	103	94	60	43	31	37	63	78	97	108	853
62	None	Pyrite	533	896	615	381	308	159	105	110	207	168	141	151	3,774
63	CaO	3,750	Pyrite	865	1,111	643	535	380	298	215	171	393	298	307	280	5,496
64	MgO	3,750	Pyrite	297	242	161	159	101	65	56	47	110	104	104	101	1,547
65	CaO	32T	Pyrite	4,951	1,724	1,276	1,503	1,161	1,160	954	868	979	933	928	1,116	17,553
66	MgO	32T	Pyrite	75	63	88	92	56	42	35	39	57	78	109	127	861

67	None	Sulfur	1,280	617	400	306	186	128	89	103	190	149	140	137	3,725
68	CaO	3,750	Sulfur	2,310	907	384	345	234	179	137	142	296	241	230	212	5,617
69	MgO	3,750	Sulfur	526	214	245	189	119	83	72	67	124	95	109	113	1,956
70	CaO	32T	Sulfur	4,163	1,973	1,772	1,864	1,342	1,050	660	612	858	859	1,001	1,115	17,269
71	MgO	32T	Sulfur	55	65	72	102	59	40	35	33	54	79	109	136	839
Rainfall content.....				36	48	29	45	31	21	30	53	40	444†
Rainfall.....				41	51	55	56	51	55	44	35	51	59	55	58	611
Average.....																51

* Pounds or tons CaO \approx for each 2,000,000 pounds of soil, moisture-free basis.

† On the basis of an average of 9 of the 12 years.

enhancement in outgo, however, was equivalent to only 18.1 per cent of the addition. A good part of the outgo of Ca + Mg may be attributed to the calcium and magnesium that is added through rainfall—computed to be 808 pounds, or 25 per cent of the loss from the untreated soil. If the determined outgo of 3,257 pounds from the untreated soil be corrected for the 808-pound rainfall increment, the net loss is 2,449 pounds, or 204 pounds yearly. In each comparison of CaO and MgO the greater solubility of the magnesium complex was responsible for a consistently greater periodic and total Ca + Mg outgo, in which was masked the decidedly repressive effect of magnesium upon calcium outgo (7).

The outgo from the limed soils may be logically assumed to have been derived mainly from the freshly formed and more soluble absorption complexes. If it be true that the entire calcium-magnesium outgo was due to the liming materials, and that the original calcium-magnesium content continued in the original state, it would mean that, as an average, 92.3 per cent of the added materials—

TABLE 4

The conservation of Ca + Mg from single economic additions of burnt lime and 100-mesh limestone and dolomite, as measured by the outgo over a 12-year period—terms of $\text{CaCO}_3 \approx$ per 2,000,000 pounds of soil*

OUTGO OF Ca + Mg, AS $\text{CaCO}_3 \approx$	FROM UNTREATED SOIL	FROM CaO ADDITION	FROM LIMESTONE ADDITION	FROM DOLOMITE ADDITION
Actual.....pounds	3,257	3,892	3,712	3,934
Increase.....pounds	635	455	677
Increase.....per cent of addition	17.8	12.7	18.

* 2,000-pound CaO \approx , or 3,570-pound $\text{CaCO}_3 \approx$.

burnt lime, limestone, and dolomite—had been lost by leaching during the 12-year period. To a certain extent this may be true, but it could not be the absolute condition in the case of the burnt lime and limestone, because the leached magnesium was derived almost entirely from the soil's original supply, plus precipitation, in both limed and unlimed soil, in spite of the "reciprocally repressive" effect. If the outgo of magnesium from the untreated soil and that from the limed soil be considered as identical, and all of the calcium loss be considered as derived from the added lime, then the recovery of calcium from the burnt lime would be 86 per cent of the addition. A similar calculation would give an 85 per cent loss from the 100-mesh limestone. Since the dolomite gave a calcium outgo of less than that from the untreated soil, the effect exerted by absorbed magnesium was probably to conserve the original calcium; therefore, the calcium outgo from the dolomite treatment may be attributed in most part to the calcium content of the dolomite.

Sulfur-addition groups. The relationships found for the total outgo of both

calcium and magnesium from the triplicated additions of lime and magnesia are such that the three sulfur groups may well be considered together. The total enhancement in calcium outgo was comparable for the three unsupplemented sulfur materials. It is interesting to observe, however, that the pyrite addition gave an outgo of calcium in excess of that from the ferrous sulfate and that from sulfur during each of the last 11 annual periods. The order of calcium outgo was the same for each sulfur material—heavy lime, light lime, sulfur material, light magnesia, and heavy magnesia additions. In like manner there was a constant order of magnesium outgo for all three groups—heavy magnesia, light magnesia, sulfur material, light lime, and heavy lime additions. On the whole, the total calcium, or magnesium, outgo from each liming material was uniform, irrespective of the form in which sulfur was added. The one notable exception was the smaller outgo of calcium from the heavy addition of burnt lime with ferrous sulfate, as compared with larger and concordant losses from the heavy addition with supplements of sulfur and pyrite.

A previous paper (7) shows the repressive effect that magnesium exerted upon calcium outgo in the unsulfured soil. It also shows that the unsupplemented sulfur materials caused a uniform increase in the outgo of calcium from the native supplies. It is strikingly evident that the magnesian additions protected the native calcium against the action of the sulfate radical, so that the calcium outgo from each sulfur material with a magnesia supplement was less than that from the untreated soil. In this respect the heavier additions of magnesia were consistently more effective. This means that even the 1-ton MgO addition prevented any interchange between the exchangeable calcium of the soil and the magnesium content of the 3,750-pound magnesium sulfate increments. In figure 1 is shown the corresponding repressive and protective tendency of liming materials upon magnesium outgo. The single exception found was the pyrite with the 1-ton addition of burnt lime. In this case, where the sulfates were generated slowly over a long period, the magnesium outgo was approximately that from the untreated soil.

Potassium outgo

The potassium outgo may be discussed briefly as a whole. All of the light unsupplemented, calcic and magnesian materials caused some depression in the total outgo of potassium (table 5). Neither of the unsupplemented sulfur-carriers induced a positive liberation of potassium in the acid soil, in spite of the bathing action of neutral salts of calcium and magnesium that were supplied immediately in one case and progressively in two cases. When used with the three sulfur materials, each addition of lime and of magnesia caused a definite decrease in potash outgo, the heavier additions being consistently more effective. There is a remarkable agreement in the potassium leachings obtained from each liming material, at each rate, when used with the three sulfur materials.

OUTGO OF NITRATE

The annual and total losses of nitrate nitrogen are given in table 6. Five of the six liming treatments without sulfur caused some increase in the outgo of nitrogen. The losses induced by the 3,750-pound additions were somewhat

TABLE 5

The influence of various forms and amounts of calcic and magnesian materials and three sulfur supplements upon the outgo of the native potash of a loam soil over a 12-year period

TANK NUM- BER	LIME OR MAGNESIA ADDITIONS		SULFUR ADDITION†	ANNUAL OUTGO OF K IN POUNDS, FROM 2,000,000 POUNDS OF SOIL												
	Form	Rate*		First	Second	Third	Fourth	Fifth	Sixth	Seventh	Eighth	Ninth	Tenth	Eleventh	Twelfth	Total 12 years
50	None	None	11.8	8.4	12.7	7.0	6.2	5.5	3.6	6.3	11.5	10.6	8.3	9.0	101
51	L.S.	2,000	None	9.0	7.0	9.0	9.5	7.8	3.1	4.0	6.3	10.1	13.5	6.3	9.0	95
52	Dol.	2,000	None	7.4	5.5	9.6	8.5	4.1	3.8	4.4	4.8	6.8	5.0	12.4	6.7	79
53	CaO	2,000	None	9.2	6.7	10.2	11.2	4.7	4.5	4.3	6.2	8.7	4.9	11.4	4.8	87
54	CaO	3,750	None	5.6	6.8	11.6	7.5	4.1	3.6	2.4	5.6	9.9	13.1	10.1	7.0	87
55	MgO	2,000	None	7.7	6.1	8.5	5.1	4.4	2.5	4.5	6.7	9.8	7.0	4.0	6.3	73
56	MgO	3,750	None	8.5	6.3	9.8	8.9	4.2	3.2	4.2	8.6	10.1	5.0	4.8	9.0	83
57	None	FeSO ₄	14.8	10.2	9.6	12.2	6.6	5.4	4.0	5.8	6.8	9.4	5.4	9.0	99
58	CaO	3,750	FeSO ₄	6.0	7.3	10.2	7.7	4.5	2.9	3.0	3.1	9.7	8.2	5.2	6.2	74
59	MgO	3,750	FeSO ₄	5.3	5.7	15.4	8.3	3.3	3.5	4.7	5.0	10.1	7.6	4.3	5.3	79
60	CaO	32T	FeSO ₄	5.4	5.3	14.6	5.5	4.9	2.3	3.0	2.2	9.8	10.6	5.4	7.9	77
61	MgO	32T	FeSO ₄	10.3	4.4	6.9	4.2	2.3	0.6	3.7	12.6	7.8	5.5	3.3	7.0	69
62	None	Pyrite	11.6	10.6	11.3	10.8	6.6	5.3	2.2	4.0	14.3	6.8	8.2	10.6	102
63	CaO	3,750	Pyrite	6.5	10.8	8.1	7.5	5.2	2.8	2.4	2.8	8.0	4.8	4.0	8.4	71
64	MgO	3,750	Pyrite	8.7	6.0	9.5	3.5	2.5	2.3	3.3	5.1	12.4	4.9	3.5	4.9	67
65	CaO	32T	Pyrite	6.4	7.0	9.6	8.8	5.1	2.9	4.0	2.6	7.5	7.0	3.7	5.8	70
66	MgO	32T	Pyrite	8.2	4.6	7.6	4.5	2.4	0.8	4.3	2.5	9.6	4.9	3.4	10.0	63
67	None	Sulfur	15.1	11.1	14.7	9.4	5.3	5.3	4.4	8.6	8.8	8.3	6.7	7.2	105
68	CaO	3,750	Sulfur	6.6	10.7	10.4	8.9	5.2	1.6	3.9	3.6	10.9	5.5	2.9	6.9	77
69	MgO	3,750	Sulfur	12.7	7.7	8.6	7.6	3.5	1.7	4.0	3.6	9.1	6.2	3.2	6.2	74
70	CaO	32T	Sulfur	8.4	6.5	7.7	11.0	6.0	3.2	3.3	2.9	7.8	5.2	6.2	7.2	75
71	MgO	32T	Sulfur	8.0	3.5	7.4	3.6	2.3	1.5	3.1	6.0	7.4	4.9	2.8	8.9	59
Rainfall..... inches				41	51	55	56	51	55	44	35	51	59	55	58	611
Average.....																51

* Pounds or tons, CaO = to each 2,000,000 pounds of soil, moisture-free basis.

† Constant rate of 1,000 pounds of S to 2,000,000 pounds of soil, moisture-free basis.

greater than those from the 2,000-pound CaO-equivalent additions, and magnesia tended to give a greater outgo than did lime.

The outgo from each unsupplemented sulfur material was less than that from the untreated soil. The ferrous sulfate control gave the minimum for

the 22 tanks. Each light addition of lime and magnesia gave an increased outgo of nitrates, when used with each of the three sulfur materials. In each of the pairs of CaO and MgO at the 1-ton-plus rate, the magnesia gave the greater outgo. A reverse relationship was obtained in a similar com-

TABLE 6

The 12-year outgo of nitrate nitrogen from a loam soil as influenced by 1,000-pound S-equivalent additions of ferrous sulfate, pyrite, and sulfur, with and without lime and magnesia

TANK NUMBER	LIME OR MAGNESIA ADDITIONS		SULFUR ADDITIONS	ANNUAL OUTGO OF NITRATE N IN POUNDS, FROM 2,000,000 POUNDS OF SOIL												
	Form	Rate*		First	Second	Third	Fourth	Fifth	Sixth	Seventh	Eighth	Ninth	Tenth	Eleventh	Twelfth	Total 12 years
50	None	None	24	49	15	23	20	17	18	8	34	11	18	16	253
51	L.S.	2,000	None	21	63	27	33	14	13	21	11	30	14	17	20	284
52	Dol.	2,000	None	20	54	20	28	14	9	13	8	32	5	15	13	231
53	CaO	2,000	None	24	58	25	29	15	19	20	13	33	5	14	13	268
54	CaO	3,750	None	40	72	35	19	16	17	14	11	34	13	21	20	312
55	MgO	2,000	None	34	55	27	25	18	20	16	13	31	7	19	17	282
56	MgO	3,750	None	41	66	35	39	16	16	17	12	32	5	20	23	322
57	None	..	FeSO ₄	13	37	16	17	12	10	12	11	31	9	19	11	198
58	CaO	3,750	FeSO ₄	22	49	25	23	19	14	20	14	33	8	17	16	260
59	MgO	3,750	FeSO ₄	23	46	30	28	22	22	18	18	35	8	16	18	284
60	CaO	32T	FeSO ₄	15	129	111	59	32	18	11	15	40	11	24	17	482
61	MgO	32T	FeSO ₄	26	145	70	35	25	27	20	23	29	5	26	20	451
62	None	..	Pyrite	17	28	17	20	24	19	14	17	30	7	18	16	227
63	CaO	3,750	Pyrite	46	55	26	28	15	24	14	11	37	5	16	14	291
64	MgO	3,750	Pyrite	51	61	27	30	18	21	16	14	38	5	17	16	314
65	CaO	32T	Pyrite	24	125	108	99	49	27	17	12	34	7	21	19	542
66	MgO	32T	Pyrite	35	106	46	32	24	44	26	22	30	5	23	20	413
67	None	..	Sulfur	13	34	16	20	16	17	16	15	36	8	17	12	220
68	CaO	3,750	Sulfur	22	45	24	27	16	16	16	16	35	5	23	15	260
69	MgO	3,750	Sulfur	30	52	21	28	18	18	15	13	36	6	20	15	272
70	CaO	32T	Sulfur	18	136	75	103	49	35	18	14	38	5	24	16	531
71	MgO	32T	Sulfur	22	141	49	40	26	37	26	20	30	5	25	28	449
Rainfall inches				41	51	55	56	51	55	44	35	51	59	55	58	

* Pounds or tons, to each 2,000,000 pounds of soil, moisture-free basis.

parison of the 32-ton additions. The intensive alkalinity of the heavy lime treatment produced a partial sterility during most of the initial year; but during the second, third, and fourth years the recovery was very marked. During the first year, two of the first four percolate collections from each 32-ton addition of lime were *devoid* of nitrates. In each case the amount of

nitrogen that passed out between the beginning of the experiment, August 3, 1917, and April 22, 1918, was less than 1 pound. The nitrate-nitrogen leached out between April 22, and the first birthday, August 3, 1918, therefore amounted to approximately 14, 23, and 17 pounds for the 32-ton CaO additions with ferrous sulfate, pyrite, and sulfur, respectively. The initial effect of the MgO was not repressive, nor did it show a subsequent accelerative effect as great as that shown by the heavy lime addition. It may be concluded that a sulfur addition will be apt to cause a reduction in the generation of nitrates, but that this effect is eliminated when moderate additions of lime and magnesia are also made.

SUMMARY

The oxidation of 1,000-pound sulfur additions of pyrite and elemental sulfur, as influenced by lime and magnesia, was studied with ferrous sulfate control in a 12-year lysimeter experiment without subsoil, through determination of the outgo of sulfates, calcium, magnesium, potassium, and nitrates.

Burnt lime, magnesia, limestone, and dolomite gave increases in sulfate outgo, but no 2,000-pound calcium oxide equivalent addition gave a sulfate outgo equal to the sulfates supplied by rainfall.

Sulfate outgo from ferrous sulfate additions corresponded to that from sulfur in the order of heavy magnesia, light magnesia, light lime, sulfur material alone, and heavy lime. The order of outgo from the pyrite was light lime, pyrite alone, heavy lime, light magnesia, and heavy magnesia. The divergent effect of lime and magnesia indicated that the organism that induced oxidation of organic soil sulfur and elemental sulfur was different from the one that produced sulfates from pyrite. The chemical oxidation of pyrite was also considered.

Complete recoveries of the sulfate radical were obtained from the ferrous sulfate additions with light calcium and magnesium oxides and heavy magnesium oxide supplements, but not from the sulfate alone or sulfate plus 32 tons of lime. Complete sulfate-equivalences of the elemental sulfur were obtained from the two magnesia supplements, but not from the sulfur control, or the sulfur addition with lime supplements. Complete recovery was not obtained from any one of the five pyrite additions. The formation of insoluble sulfates and the reduction of sulfates are considered as factors responsible for incomplete sulfate recoveries.

The increases in the outgo of calcium and magnesium from the unsupplemented liming materials were considered in connection with absorbed and natural bases, as affecting the persistence of the additions and the protection afforded native materials.

The total losses of calcium and magnesium from the several additions were generally in close agreement, irrespective of the form of the added sulfur, although the periodic outgo was materially influenced by the nature of the sulfur addition.

Each of the six single liming treatments effected a decrease in the outgo of potassium, and no unsupplemented sulfur material produced a positive increase in the outgo of potassium from the acid soil. Each lime and magnesia addition produced a marked decrease in potassium outgo from the several combinations with sulfur materials, the heavier additions being more active in their repressive effect.

Some increase in total nitrate outgo was induced by the single additions of liming materials. Each of the unsupplemented sulfur materials produced a decrease in nitrate outgo, but the lime and magnesia supplements overcame this tendency. The light additions of magnesia caused a greater nitrate outgo than did the light lime supplements. The reverse was true for the heavy additions, in spite of an absolute initial inhibition by the 32-ton calcium oxide supplements.

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THE LAWS OF SOIL COLLOIDAL BEHAVIOR: III. ISOELECTRIC PRECIPITATES¹

SANTE MATTSON

New Jersey Agricultural Experiment Station

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Soil colloidal materials divide themselves electrokinetically into positive and negative colloids. Most soil colloids consist of silica, humus, and the sesquioxides of iron and aluminum. Silica and humus are strictly electronegative. They adsorb and combine with bases and are, according to the terminology of Michaelis (12), acidoid colloids. The sesquioxides are electrical ampholytes, being electropositive in acid and electronegative in alkaline solutions. The position of their isoelectric point with respect to the pH depends upon the nature of the acid anion which always enters into the composition in their electropositive condition. The sesquioxides adsorb both acids and bases and are therefore ampholytoid colloids.

Since positive and negative sols mutually flocculate and electrically neutralize each other, forming new combinations, the properties of which vary with the relative proportion of each component, it is obviously of the greatest importance to learn something about the laws governing such reactions. In order to understand the soil forming processes it is first necessary to know something about the behavior of the soil colloids, for it is these, which, by flocculation and deposition in one layer and by dispersion and migration to another layer, give rise to the development of soil horizons.

In a previous paper (9) a series of alumino-silicates, isoelectrically precipitated, were studied. In the present work this study has been extended to include other important combinations which can be isoelectrically precipitated. The isoelectric precipitates prepared and studied in this work include: iron and aluminum silicates, humates, phosphates, and hydroxides, together with various mixtures of these combinations.

METHOD

Accurately measured portions of the two solutions, A and B, to be mixed were placed in separate beakers and sufficient water was added to make a combined volume of 50 cc. The mixing was rapidly done by pouring the content of one of the beakers into the other and then back into the first, then again into the second, and finally into a large stoppered pyrex test tube.

¹ Journal series paper of the New Jersey Agricultural Experiment Station, Department of Soil Chemistry and Bacteriology.

The precipitating zone was found by a preliminary test by running one of the solutions into the other from a burette until a rapid flocculation was observed. This zone was then covered by a series of tubes prepared as explained, either by varying the volume of one of the two solutions or, when the proportions of the solutions were kept constant, by changing the pH by adding HCl or NaOH as the case required. The HCl was always added to the iron and aluminum solutions and the NaOH to the silicate, humate, and phosphate solutions before these solutions were mixed.

The flocculations after the solutions were mixed and after they were allowed to stand over night are recorded in the tables. The latter is represented by crosses, x signifying slight, xx about half complete, xxx nearly complete, and xxxx complete flocculation. The tubes were then shaken and the cataphoresis measurements made as described elsewhere (10). The pH was determined colorimetrically.

From the isoelectric proportions thus found, either directly or by interpolation of the migration velocities, a larger quantity, usually 2.5 liters, was prepared by mixing the same proportions of the various solutions in exactly the same manner. Since the liquid cannot be removed from the floc without disturbing the equilibrium, the composition of the floc was determined by analyzing the supernatant liquid. The Cl and SO_4 ions in the floc were determined by subtracting the quantity found in a measured volume of supernatant liquid from the quantity found in the same volume of liquid which contained the floc.

ISOELECTRIC "HYDROXIDES" OF IRON AND ALUMINUM

The electrokinetic behavior at various degrees of alkalization and the composition of the isoelectric precipitates of the "hydroxides" of aluminum and iron are shown in tables 29-32.

We speak about electropositive iron and aluminum hydroxides, thereby implying that the definite compounds $\text{Fe}(\text{OH})_3$ and $\text{Al}(\text{OH})_3$ carry a positive charge and migrate to the cathode when, as a matter of fact, these materials are electronegative. It is well known that positive iron and aluminum "hydroxides" contain a certain amount of acid anions. The more acid the medium the greater the quantity of anions in the complex and the more strongly electropositive it is, until the complex resolves itself into a dispersion of single ions. The colloid chemist cannot rightly speak about his colloidal complexes in terms of definite compounds whether he is dealing with negative arsenious sulfide or positive iron hydroxide. A definite compound implies a stoichiometric relationship between the constituent parts. In a colloidal complex there is no such definite relationship, which exists probably only in the interior of the crystal lattice. At the interfaces the free valences attract the various ions present in the solution and these ions combine with the surface layer in proportion as their solution tension and hydration decreases. BaSO_4 is positive with an excess of Ba ions and negative with an

TABLE 29
The $\text{AlCl}_3 + \text{NaOH}$ —System No. 3

A. 5.0 millimols AlCl_3 in 1,000 cc.
B. 20.0 millimols NaOH in 1,000 cc.

SOLUTION A	SOLUTION B*	FLOCCULATION		$\mu/\text{SEC.}$ 1 VOLT/CM.	pH
		After mixing	Overnight		
cc.	cc.				
20	14.5	Clear	0
20	15.0	Instant	xxxx	+1.38	...
20	15.5	Instant	xxxx	+0.61	7.7
20	16.0	Instant	xxxx	± 0.0	8.1
20	16.5	Instant	xxxx	-0.47	8.4
20	17.0	Instant	xxxx	-0.67	...
20	17.5	Rapid	xxxx	-1.01	...
20	18.5	Clear	x

* Plus water to make 30 cc. or a total volume of 50 cc.

Isoelectric mixture:

5.0 millimols AlCl_3 in 1,000 cc.

16.0 millimols NaOH in 1,500 cc.

pH 8.0.

Composition of floc: $(\text{Al}_2\text{O}_3)_{416} \cdot \text{Al}_2\text{O}_3\text{Cl}_2$ or 0.0048 equivalent Cl per mol Al_2O_3 .

TABLE 30
The $\text{FeCl}_3 + \text{NaOH}$ —System No. 4

A. 5.0 millimols FeCl_3 in 1,000 cc.
B. 20.0 millimols NaOH in 1,000 cc.

SOLUTION A	SOLUTION B	FLOCCULATION		$\mu/\text{SEC.}$ 1 VOLT/CM.	pH
		After mixing	Overnight		
cc.	cc.				
20	14.5	Clear	0
20	14.75	Opal	xxxx	+2.53	6.2
20	14.88	Instant	xxxx	+1.29	6.5
20	15.0	Instant	xxxx	+1.04	6.75
20	15.12	Instant	xxxx	+0.93	6.9
20	15.25	Instant	xxxx	± 0.0	7.1
20	15.38	Instant	xxxx	-0.48	7.2
20	15.5	Instant	xxxx	-1.08	7.55
20	15.75	Clear	xxxx	-3.20	8.2

Isoelectric mixture:

5.0 millimols FeCl_3 in 1,000 cc.

15.25 millimols NaOH in 1,500 cc.

pH 7.15.

Composition of floc: $(\text{Fe}_2\text{O}_3)_{387} \cdot \text{Fe}_2\text{O}_3\text{Cl}_2$ or 0.0052 equivalent Cl per mol Fe_2O_3 .

TABLE 31
The $Al_2(SO_4)_3 + NaOH$ —System No. 2

A. 2.5 millimols $Al_2(SO_4)_3$ in 1,000 cc.

B. 20.0 millimols NaOH in 1,000 cc.

SOLUTION A	SOLUTION B	FLOCCULATION		μ /SEC. 1 VOLT/CM.	pH
		After mixing	Overnight		
cc.	cc.				
20	5.0	Slow	xx	+0.87	...
20	7.5	Rapid	xx
20	10.0	Instant	xxxx	+0.87	...
20	14.0	Instant	xxxx	+0.55	...
20	14.5	Instant	xxxx	+0.38	7.3
20	15.0	Instant	xxxx	± 0.0	7.6
20	15.5	Instant	xxxx	-0.76	8.0
20	16.5	Instant	xxxx	-1.01	...
20	17.5	Rapid	xxx
20	18.5	Slow	xx	-2.33	...

Isoelectric mixture:

2.5 millimols $Al_2(SO_4)_3$ in 1,000 cc.

15.0 millimols NaOH in 1,500 cc.

pH 7.5.

Composition of floc: $(Al_2O_3)_{12.6} \cdot Al_2O_3SO_4$ or 0.0737 mol SO_4 per mol Al_2O_3 .

TABLE 32
The $Fe_2(SO_4)_3 + NaOH$ —System No. 1

A. 2.5 millimols $Fe_2(SO_4)_3$ in 1,000 cc.

B. 20.0 millimols NaOH in 1,000 cc.

SOLUTION A	SOLUTION B	FLOCCULATION		μ /SEC. 1 VOLT/CM.	pH
		After mixing	Overnight		
cc.	cc.				
20	5.0	Slow	xx	+0.32	...
20	7.5	Rapid	xxx	+0.30	...
20	10.0	Instant	xxxx	+0.30	...
20	14.0	Instant	xxxx	+0.47	...
20	14.5	Instant	xxxx	+0.47	...
20	15.0	Instant	xxxx	+0.61	6.4
20	15.5	Instant	xxxx	+0.40	7.0
20	16.0	Rapid	xxxx	-1.78	7.8
20	16.5	Slow	xxxx	-2.75	...

Isoelectric mixture:

2.5 millimols $Fe_2(SO_4)_3$ in 1,000 cc.

15.59 millimols NaOH in 1,500 cc.

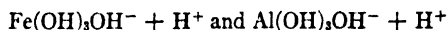
pH 7.05.

Composition of floc: $(Fe_2O_3)_{73} \cdot Fe_2O_3SO_4$ or 0.0135 mol SO_4 per mol Fe_2O_3 .

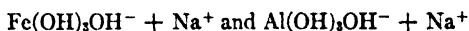
excess of SO_4 ions in the solution (10). AgI is positive with an excess of Ag ions and negative with an excess of I ions in the solution (6). The more highly dispersed these materials are, that is, the greater the surface exposed and the more electropositive or electronegative they are, that is, the greater the excess of a cation or anion in the adsorption layer, the more their composition will depart from a stoichiometric relationship. Hence the "occlusion" spoken of by the analytical chemist.

The composition of a colloidal complex varies constantly with several factors, such as the pH, the composition and concentration of the dispersing solution, and the temperature. Its composition is also influenced by the manner of mixing the reacting solutions, as we shall see. It is also affected by time, as shown by the phenomenon of aging and by a drift in the isoelectric point which is always observed. There must be a balance within the micelle between the positive and negative ions but a stoichiometric relationship between any pair of ions must be accidental.

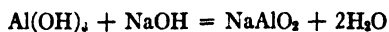
Returning now to a discussion of the tables, we note, that not even the isoelectric "hydroxides" of iron and aluminum are purely hydroxides. They still retain some of the acid anions. This might be explained as follows: In general all inert substances charge themselves negatively in water. This may be accounted for by assuming the electrical forces within the oriented, interfacial layers of molecules to be such as to attract and fix the OH ions of the water while the H ions (or any other cation which might have displaced the H ions) remain free and diffused into the surrounding water, which then assumes a corresponding positive charge. The resulting complex in the case of negative iron and aluminum hydroxides may be represented thus:



In a NaOH solution these materials, like all others, become strongly electro-negative, as the following formula would explain:

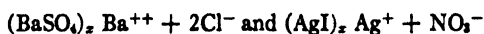


Both hydroxides adsorb quantities of the base in alkaline solutions. The combination formed is commonly assumed to be in the form of an aluminate, thus:



There is, however, no proof of this. The combination is more likely to be in the form of a simple addition such as $\text{NaAl}(\text{OH})_4$. This seems to be the way bases combine with soil colloids (9).

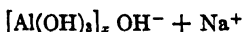
Substances which charge themselves positively in water do so by adsorbing an excess of the cations, thus:



or by dissociating a diffusible anion as the oxychlorides of the sesquioxides, thus:



To return to the question, if the iron and aluminum compounds are negative in this form:

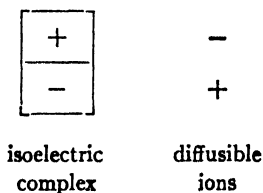


and positive in this:



then it is evident that the isoelectric complex must still retain some of the acid anions, for otherwise it would be negative.

The isoelectric point is, according to Michaelis (13) that point at which the ampholyte dissociates an equal number of anions and cations. This would of course result in an isoelectric amphoteric complex as represented by the following scheme.



Such an isoelectric ion complex is electrically compensated internally. There is, therefore, nothing to prevent the diffusible ions from diffusing into the surrounding medium, since under these conditions they are always paired. There can, therefore, be no ion atmosphere surrounding an isoelectric particle. This leads to the following important consequences:

The swelling, the viscosity, the osmotic pressure, and the Donnan potential are all at a minimum at the isoelectric point, as found by Loeb in the case of the proteins (5).

The "purification" of a colloid, i.e. the removal of diffusible ions, is most readily accomplished at the isoelectric point.

It is interesting to note that considerably more of the SO_4 ion than of the Cl ion is retained at the isoelectric point. This shows that the SO_4 ion is much less dissociated by the complex than is the monovalent Cl ion. This is in harmony with the observation, reported elsewhere (8), that the amphoteric Aragon soil colloid adsorbed 0.269 milliequivalents SO_4 and only 0.044 m. e. Cl per gram from solutions of the respective acids, but was much more strongly electropositive in the HCl than in the H_2SO_4 solution, the corresponding migration velocities toward the cathode being 0.6 and 2.8μ per second respectively.

TABLE 33

*The $AlCl_3 + Na_2HPO_4$ —System No. 5*A. 4.96 millimols $AlCl_3$ in 1,000 cc.B. 10.228 millimols Na_2HPO_4 in 1,000 cc.

SOLUTION A	SOLUTION B	FLOCCULATION		μ /SEC. 1 VOLT/CM.	pH
		After mixing	Overnight		
cc.	cc.				
20	18.0	Opal	xxxx	+1.78	4.0
20	19.0	Slow	xxxx	+0.92	4.3
20	19.5	Rapid	xxxx
20	20.0	Rapid	xxxx	-0.15	5.0
20	20.5	Rapid	xxxx	-0.67	5.4
20	21.0	Rapid	xxxx	-0.98	5.7
20	22.0	Slow	xxxx	-1.30	5.95

Isoelectric mixture:

4.96 millimols $AlCl_3$ in 1,000 cc.10.156 millimols Na_2HPO_4 in 1,500 cc.

pH 4.9.

 PO_4 in solution: 5.904 millimols. PO_4 combined: 4.252 millimols.Composition: $Al_2O_3 \cdot (P_2O_5)_{0.857}$.

TABLE 34

*The $FeCl_3 + Na_2HPO_4$ —System No. 6*A. 5.0 millimols $FeCl_3$ in 1,000 cc.B. 10.228 millimols Na_2HPO_4 in 1,000 cc.

SOLUTION A	SOLUTION B	FLOCCULATION		μ /SEC. 1 VOLT/CM.	pH
		After mixing	Overnight		
cc.	cc.				
20	13.0	Opal	xxxx	+1.68	...
20	15.0	Instant	xxxx	+1.16	3.3
20	17.0	Instant	xxxx	+0.67	3.5
20	18.0	Instant	xxxx	+0.38	3.8
20	18.5	Instant	xxxx	+0.10	4.0
20	19.0	Instant	xxxx	-0.43	4.2
20	19.5	Instant	xxxx	-0.58	4.4
20	21.0	Opal	xxx	-1.68	5.0

Isoelectric mixture:

5.0 millimols $FeCl_3$ in 1,000 cc.9.512 millimols Na_2HPO_4 in 1,500 cc.

pH 4.0.

 PO_4 in solution: 4.943 millimols. PO_4 combined: 4.569 millimols.Composition: $Fe_2O_3 \cdot (P_2O_5)_{0.914}$.

TABLE 35
The $AlCl_3 + Na_3PO_4$ —System No. 7
 A. 5.0 millimols $AlCl_3$ in 1,000 cc.
 B. 5.114 millimols Na_3PO_4 in 1,000 cc.

SOLUTION A	SOLUTION B	FLOCCULATION		μ/SEC 1 VOLT/CM.	pH
		After mixing	Overnight		
cc.	cc.				
20	19.0	Opal	0
20	20.0	Opal	xx
20	20.5	Slow	xxxx	+1.32	...
20	21.0	Rapid	xxxx	+0.50	5.5
20	21.5	Rapid	xxxx	-0.25	5.7
20	22.0	Rapid	xxxx	-0.70	...
20	22.5	Rapid	xxxx	-1.08	...
20	23.0	Slow	xxxx	-1.10	6.4
20	24.0	Opal	xxxx
20	25.0	Opal	xxx

Isoelectric mixture:

5.0 millimols $AlCl_3$ in 1,000 cc.

5.482 millimols Na_3PO_4 in 1,500 cc.

pH 5.6.

PO_4 in solution: 1.639 millimols.

PO_4 combined: 3.843 millimols.

Composition: $Al_2O_3 \cdot (P_2O_5)_{0.769}$.

TABLE 36
The $AlCl_3 + Na_3PO_4 + NaOH$ —System No. 8
 A. 5.0 millimols $AlCl_3$ in 1,000 cc.
 B. 5.114 millimols Na_2HPO_4 } in 1,000 cc.
 20.0 millimols NaOH }

SOLUTION A	SOLUTION B	FLOCCULATION		$\mu/SEC.$ 1 VOLT/CM.	pH
		After mixing	Overnight		
cc.	cc.				
20	9.4	Opal	0	4.6
20	9.5	Opal	0	5.1
20	9.6	Slow	xxxx	+1.38	6.0
20	9.8	Instant	xxxx	-0.38	6.5
20	10.0	Rapid	xxxx	-1.08	6.8
20	10.2	Rapid	xxxx	-1.32	6.9
20	10.4	Slow	xxxx	-1.68	7.0
20	10.5	Opal	xx	7.1

Isoelectric mixture:

5.0 millimols $AlCl_3$ in 1,000 cc.

2.495 millimols Na_2HPO_4 } in 1,500 cc.
 9.76 millimols NaOH }

pH 6.45.

PO_4 in solution: 0.085 millimol.

PO_4 combined: 2.410 millimols.

Composition: $Al_2O_3 \cdot (P_2O_5)_{0.482}$.

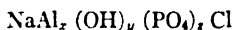
The alumina retains nearly six times as much SO_4 as the iron hydroxide although the alumina is isoelectric at a higher pH. This is in agreement with the observation of analytical chemists that the SO_4 ion is very difficult to remove from the $\text{Al}(\text{OH})_3$ precipitate. The OH ion displaces the acid anion more readily in the iron than in the aluminum compound.

The effect of the free divalent sulfate ions is seen in the greatly extended flocculating zone on the positive side. In the case of the sulfates the floc begins to form after the addition of 5 cc. of the alkali solution, whereas the chlorides remain stable up to about 15 cc. of the alkali. This influence is also reflected in the migration velocities. The chloride systems become strongly positive and negative, whereas the sulfate systems attain a high charge only on the negative side.

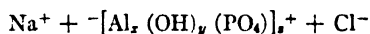
ISOELECTRIC IRON AND ALUMINUM "PHOSPHATES"

Tables 33-36 show the isoelectric precipitation and the composition of the floc in a series of three "phosphates" of aluminum and one of iron.

In the first place it is evident that the isoelectric "phosphates" are not pure phosphates. Every ion in the system enters to some extent in the combination. Besides the Al (or Fe), the OH , and the PO_4 ions, which together make up the colloidal complex, there is at the isoelectric point some Cl ions and some Na ions. The complex may be represented by the formula



which by dissociation would yield



which would be isoelectric, since the anionic and cationic dissociation balance each other.

This complex is isoelectric at a lower pH than the "hydroxide." The reason for this is that the Cl ions, which persist in the "hydroxide" at a pH above 7.0, are actively displaced by the phosphate ions while the solution is still acid.

The fact that the isoelectric point is displaced far over on the acid side, the displacement being greater with greater PO_4 concentration, shows that the anions $\text{HPO}_4^{''}$ and $\text{PO}_4^{'''}$ are, like the OH ions, not appreciably dissociated by the complex. The dissociation of the phosphate ions is not great enough to overcome the effect of the cationic dissociation until we reach a lower pH value. Without the phosphate ions the quantity of Cl in the complex at these pH values would be higher and the complex would, as we have seen, be strongly positive. The phosphate ions, because of their high concentration in the solution, displace the Cl ions in the complex at very much lower pH values than do the OH ions.

The solution tension of the OH ions, that is, the dissociation constant of

the hydroxide, is however, lower than that of the phosphate. In table 36, system no. 8, the pH at the isoelectric point is 6.45, which is equal to a OH ion concentration of 0.282×10^{-7} , whereas the molar phosphate concentration is $0.000085 \div 2.5 = 340.0 \times 10^{-7}$, or over 1,000 times as great, yet the complex formed is over 50 per cent basic. "Normal," or an approach to normal, phosphates of iron and aluminum can therefore only be precipitated in an acid solution with a large excess of phosphate.

As in the case of the "hydroxides" the ferric "phosphate" is isoelectric at a lower pH than the aluminum "phosphate." The ferric "phosphate" contains also a higher phosphate ratio (compare systems 5 and 6) although the phosphate-ion concentration at equilibrium was lower in the ferric system.

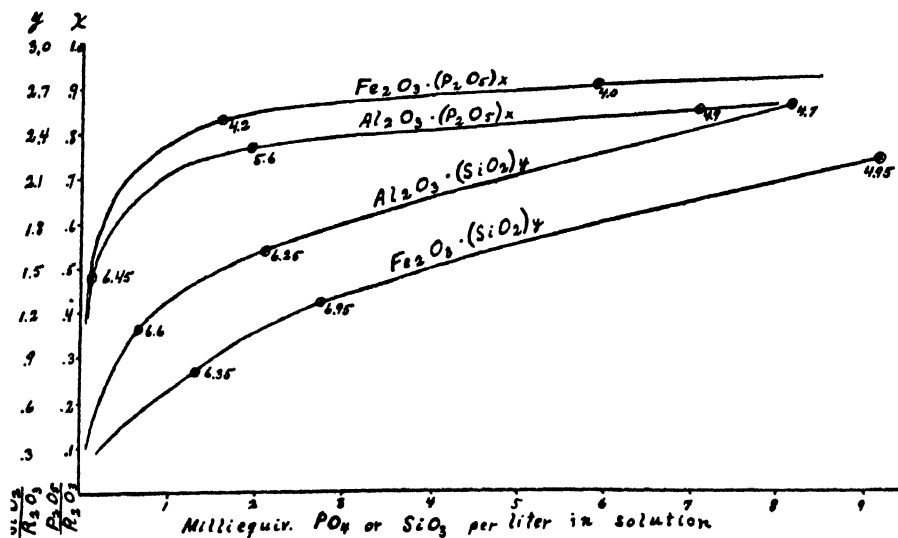


FIG. 6. THE RELATION OF THE PHOSPHATE AND SILICATE CONCENTRATION AND THE pH TO THE COMPOSITION OF THE ISOELECTRIC PRECIPITATES OF ALUMINUM AND FERRIC "PHOSPHATES" AND "SILICATES"

Scattered figures indicate pH

Both the hydroxyl and the phosphate ions are therefore more firmly associated in the ferric than in the aluminum compound. The ferric complex requires, therefore, a lower pH to retain enough Cl ions for the anionic dissociation to balance the cationic dissociation, i.e., the isoelectric condition. It will be noted that the ferric complex does not become as strongly electropositive as the aluminum complex and that the former flocculates on the positive or acid side over a much wider range. The very low positive charge observed in the sulfate-hydroxide systems (1 and 2), and ascribed to the depressing effect of the divalent SO_4 anion, is not found in the phosphate systems. The reason for this is, that on the acid side of the isoelectric point of the latter the monovalent H_2PO_4^- anion predominates.

The results of the experiments with the three aluminum chloride-phosphate mixtures show that each mixture has a different isoelectric point, the pH of which depends on the relative proportions of the two components. The higher the phosphate (the negative component) the lower is the pH at which the system is isoelectric and the greater is the ratio of phosphate to alumina in the precipitate. The higher the proportion of alumina (the positive component) in the mixture, the higher is the isoelectric pH and the lower is the ratio of phosphate to alumina in the precipitate, but the more complete is the removal of the phosphate from solution. In all this the phosphate is identical to the silicate systems previously discussed (9).

The relationship between the PO_4 combined in the isoelectric precipitates and the PO_4 concentration in the solution at equilibrium is shown in figure 6, in which we recognize the familiar "adsorption" curve.

EFFECT OF DILUTION

In order to determine the effect of dilution on the composition of the precipitates, the aluminum "phosphate" mixture no. 5 and the ferric "phosphate" mixture no. 6 were prepared in total volumes of 1 and 10 liters in addition to the isoelectric 2.5-liter systems shown in tables 33 and 34. No cataphoresis measurements were made but the precipitation was complete in all cases.

Mixture No. 5 (concentrated)

4.96 millimols AlCl_3 in 500 cc.
10.156 millimols Na_2HPO_4 in 500 cc.
pH 4.85
 PO_4 in solution: 5.670 millimols
 PO_4 combined: 4.486 millimols
Composition: $\text{Al}_2\text{O}_3 \cdot (\text{P}_2\text{O}_5)_{0.897}$

Mixture No. 6 (concentrated)

5.0 millimols FeCl_3 in 500 cc.
9.512 millimols Na_2HPO_4 in 500 cc.
pH 3.7
 PO_4 in solution: 4.656 millimols
 PO_4 combined: 4.856 millimols
Composition: $\text{Fe}_2\text{O}_3 \cdot (\text{P}_2\text{O}_5)_{0.971}$

Mixture No. 5 (diluted)

4.96 millimols AlCl_3 in 5,000 cc.
10.156 millimols Na_2HPO_4 in 5,000 cc.
pH 5.2
 PO_4 in solution: 6.205 millimols
 PO_4 combined: 3.951 millimols
Composition: $\text{Al}_2\text{O}_3 \cdot (\text{P}_2\text{O}_5)_{0.790}$

Mixture No. 6 (diluted)

5.0 millimols FeCl_3 in 5,000 cc.
9.512 millimols Na_2HPO_4 in 5,000 cc.
pH 4.2
 PO_4 in solution: 5.343 millimols
 PO_4 combined: 4.169 millimols
Composition: $(\text{Fe}_2\text{O}_3) \cdot (\text{P}_2\text{O}_5)_{0.824}$

An increase in concentration lowered the pH whereas a decrease in concentration had the opposite effect. In spite of this variation in the pH it will be seen that, if we reduce the millimols PO_4 in the solution to milliequivalents per liter and plot this value with the composition ratio as in figure 6, the points in the case of the aluminum compound will lie very nearly on the curve. The ferric "phosphate" composition ratios are higher in each case, as shown by the greater steepness of this curve.

THE COMPOSITION OF POSITIVE AND NEGATIVE FERRIC "PHOSPHATE"

The composition of the strongly negative and of the strongly positive precipitates was compared with the composition of the isoelectric precipitate

in systems containing the same proportions of the major reacting materials. Ferric "phosphate" was found to be admirably suited for this experiment because of the variations in color with the degree of saturation. The normal ferric phosphate is pale greenish gray but becomes yellowish and finally brownish as the compound becomes more basic.

By adding NaOH or HCl to the isoelectric mixture no. 6 (table 34) the system was made strongly negative or positive. The colloid, however, became more or less stable, and as a result the flocculation had to be forced by adding 500 millimols NH_4Cl . The mixtures were as follows:

Electropositive mixture No. 6a

5.0 millimols FeCl_3 + 2.5 millimols HCl in 1,000 cc.
 9.512 millimols Na_2HPO_4 + 500 millimols NH_4Cl in 1,500 cc.
 pH 3.2
 PO_4 in solution: 4.589
 PO_4 combined: 4.923
 Composition: $\text{Fe}_2\text{O}_3 \cdot (\text{P}_2\text{O}_5)_{0.985}$
 Color: pale greenish gray
 NH_4 adsorbed: 0.48 milliequivalents

Isoelectric mixture No. 6

As given in table 34
 Composition: $\text{Fe}_2\text{O}_3 \cdot (\text{P}_2\text{O}_5)_{0.914}$
 Color: pale yellowish, greenish gray

Electronegative mixture No. 6b

5.0 millimols FeCl_3 in 1,000 cc.
 9.512 millimols Na_2HPO_4 + 5.0 millimols NaOH + 500 millimols NH_4Cl in 1,500 cc.
 pH 6.5
 PO_4 in solution: 4.813 millimols
 PO_4 combined: 4.699 millimols
 Composition: $\text{Fe}_2\text{O}_3 \cdot (\text{P}_2\text{O}_5)_{0.940}$
 Color: yellowish
 NH_4 adsorbed: 2.01 milliequivalents

Electronegative mixture No. 6bb

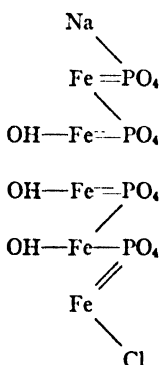
5.0 millimols FeCl_3 in 1,000 cc.
 9.512 millimols Na_2HPO_4 + 10.0 millimols NaOH + 500 millimols NH_4Cl in 1,500 cc.
 pH 7.1
 PO_4 in solution: 4.894 millimols
 PO_4 combined: 4.618 millimols
 Composition: $\text{Fe}_2\text{O}_3 \cdot (\text{P}_2\text{O}_5)_{0.924}$
 Color: brownish
 NH_4 adsorbed: 2.00 milliequivalents

The ratio of P_2O_5 to Fe_2O_3 has increased from 0.914 in the isoelectric complex to 0.985 in the positive complex. This would be expected because the positive complex was formed in a more acid solution in which OH^- ion concentration was very low. The OH^- ions offered, therefore, very little competition, resulting in a less basic complex. This was also indicated by the color, which

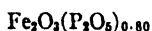
was a trace lighter in the positive floc. In the case of the negative complex we meet with an anomaly. We would anticipate this complex to be more basic in composition, as is indeed indicated by the color, which shifts to yellow and then to brown as the OH-ion concentration is increased. But the analysis reveals an increase in the composition ratio to 0.940 at a pH of 6.5 and a smaller increase to 0.924 at a pH of 7.1.

The color changes show that in the negative complexes the iron must be in actual combination with less PO_4 than in the isoelectric complex, yet the analysis shows a higher proportion in the negative complexes. We are here dealing with a colloidal complex formation which, under the conditions of the experiment, is comparatively simple. In the soil, where there is a multitude of different ions, the complex would be complicated indeed. But even here, if we are to explain what is taking place it will be necessary to free ourselves of the dogma of stoichiometry and definite proportions.

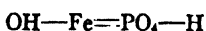
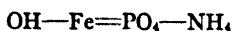
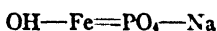
If the ferric phosphate hydrolyzes, that is, if the OH ions displace the PO_4 ions as the pH is increased, then the actual number of linkages between Fe and PO_4 is reduced but that does not necessarily mean that the complex will be poorer in PO_4 ions. In order to provide us with something tangible as a basis for our line of reasoning let the following greatly abbreviated formula represent the isoelectric complex:



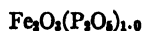
This complex would have the composition



If we now represent the electronegative complex by the following formula:



we get a combination with the following apparent composition



as if it were a normal phosphate, although this complex is in reality more basic, as far as the ferric component is concerned, than the previous one, in which the PO_4 content is lower.

If the OH ions have displaced some of the PO_4 valences then the latter must be satisfied by linking themselves with a corresponding number of cations in the solution. This was tested by a determination of the quantity of NH_4 ions which were removed from solution. It was found that whereas the electropositive precipitate adsorbed only 0.48 milliequivalents the two electronegative precipitates adsorbed 2.01 and 2.00 milliequivalents NH_4 .

This structure of the complex will explain the mechanism of ion adsorption and exchange which these phosphates possess in common with the silicates and other substances. Since this subject will be discussed further in a paper on ion adsorption and exchange we will now turn our attention to another series of isoelectric precipitates.

ISOELECTRIC "SILICATES"

Tables 37-39 show the isoelectric precipitation and the composition of the floc of aluminum and iron "silicates."

The silicate ion, like the phosphate, displaces the isoelectric point to the acid side, the displacement increasing in direct proportion to the silicate concentration in the solution and the proportion of silica which has entered into combination with the complex. The silicate ions must therefore displace the easily dissociating Cl ions. This suppresses the anionic dissociation and at the same time increases the cationic dissociation because the silicated, like the phosphated, complex adsorbs cations from the solution. For the two forms of dissociation to be equalized (the isoelectric condition) the pH must necessarily be lowered. The higher the silicate content of the complex the lower the pH at which a sufficient number of Cl ions are retained for the isoelectric condition.

The quantity of Cl ions carried down in the floc was determined in a number of the "silicate" systems. The quantities varied between 0.030 and 0.007 milliequivalent Cl per millimol Al_2O_3 or Fe_2O_3 . The rather wide variation is probably chiefly due to errors. The results show, nevertheless, that a small quantity of Cl (or any other dissociating and readily diffusible anion) is essential for the isoelectric as well as for the electropositive condition.

Since, at a given concentration and pH, less of the SiO_3 than of the PO_4 combines with the complex it is evident that the SiO_3 competes less strongly with the OH ions. This is to be ascribed to the low dissociation constant of silicic acid and possibly also to a lower solubility of the phosphate than of the silicate. The relationship is brought out more clearly in figure 6. It will be

TABLE 37A

*The $\text{AlCl}_3 + \text{Na}_2\text{SiO}_3 + \text{HCl}$ —System No. 9*A. 5.0 millimols $\text{AlCl}_3 + 22.5$ millimols HCl in 1,000 cc.B. 15.0 millimols Na_2SiO_3 in 1,000 cc.

SOLUTION A	SOLUTION B	FLOCCULATION		$\mu/\text{SEC.}$ 1 VOLT/CM.	pH
		After mixing	Overnight		
cc.	cc.				
20	21.0	Opal	xxxx	+1.12	4.35
20	21.2	Slow	xxxx	+1.08	4.35
20	21.5	Instant	xxxx	+0.82	4.4
20	22.0	Instant	xxxx	+0.40	4.5
20	22.5	Instant	xxxx	-0.76	5.0
20	23.0	Instant	xxxx	-1.44	5.65
20	24.0	Instant	xx	6.1
20	25.0	Opal	0

Isoelectric mixture: $\frac{\text{SiO}_2}{\text{Al}_2\text{O}_3} = 6.66.$

5.0 millimols $\text{AlCl}_3 + 22.5$ millimols HCl in 1,000 cc.

16.65 millimols Na_2SiO_3 in 1,500 cc.

pH 4.7.

SiO_2 in solution: 10.207 millimols.

Al_2O_3 in solution: 0.039 millimols.

Composition of floc: $\text{Al}_2\text{O}_3 (\text{SiO}_2)_{2.26}.$

TABLE 37B

*The $\text{FeCl}_3 + \text{Na}_2\text{SiO}_3 + \text{HCl}$ —System No. 10*A. 5.0 millimols $\text{FeCl}_3 + 22.5$ millimols HCl in 1,000 cc.B. 15.0 millimols Na_2SiO_3 in 1,000 cc.

SOLUTION A	SOLUTION B	FLOCCULATION		$\mu/\text{SEC.}$ 1 VOLT CM.	pH
		After mixing	Overnight		
cc.	cc.				
20	21.5	Opal	0
20	22.5	Opal	xxx	+0.84	4.2
20	23.0	Instant	xxxx	-0.80	5.3
20	23.5	Instant	xxxx	-1.68	6.1
20	24.0	Rapid	xxx	-1.78	6.4
20	24.5	Opal	x

Isoelectric mixture: $\frac{\text{SiO}_2}{\text{Fe}_2\text{O}_3} = 6.82.$

5.0 millimols $\text{FeCl}_3 + 22.5$ millimols HCl in 1,000 cc.

17.06 millimols Na_2SiO_3 in 1,500 cc.

pH 4.95.

SiO_2 in solution: 11.481 millimols.

Fe_2O_3 in solution: 0.025 millimols.

Composition of floc: $\text{Fe}_2\text{O}_3 (\text{SiO}_2)_{2.26}.$

TABLE 38A
The $\text{AlCl}_3 + \text{Na}_2\text{SiO}_3$ —System No. 11

A. 5.0 millimols AlCl_3 in 1,000 cc.

B. 7.5 millimols Na_2SiO_3 in 1,000 cc.

SOLUTION A	SOLUTION B	FLOCCULATION		$\mu/\text{SEC.}$ 1 VOLT/CM	pH
		After mixing	Overnight		
cc.	cc.				
20	17.4	Opal	0
20	17.6	Slow	xxxx	+1.38	5.7
20	17.8	Instant	xxxx	+0.76	6.1
20	18.0	Instant	xxxx	-0.67	6.4
20	18.5	Slow	xx

Isoelectric mixture: $\frac{\text{SiO}_2}{\text{Al}_2\text{O}_3} = 2.68$.

5.0 millimols AlCl_3 in 1,000 cc.

6.713 millimols Na_2SiO_3 in 1,500 cc.

pH 6.25.

SiO_2 in solution: 2.641 millimols.

Al_2O_3 in solution: 0.005 millimols

Composition of floc: $\text{Al}_2\text{O}_3(\text{SiO}_2)_{1.43}$.

TABLE 38B
The $\text{FeCl}_3 + \text{Na}_2\text{SiO}_3$ —System No. 12

A. 5.0 millimols FeCl_3 in 1,000 cc.

B. 7.5 millimols Na_2SiO_3 in 1,000 cc.

SOLUTION A	SOLUTION B	FLOCCULATION		$\mu/\text{SEC.}$ 1 VOLT/CM.	pH
		After mixing	Overnight		
cc.	cc.				
20	17.4	,Opal	0
20	17.6	Slow	xxx	+0.65	5.65
20	17.8	Instant	xxxx	-0.34	6.1
20	18.0	Instant	xxxx	-1.38	6.3
20	18.5	Rapid	xxx	-1.59	6.6

Isoelectric mixture: $\frac{\text{SiO}_2}{\text{Fe}_2\text{O}_3} = 2.66$.

5.0 millimols FeCl_3 in 1,000 cc.

6.65 millimols Na_2SiO_3 in 1,500 cc.

pH 5.95.

SiO_2 in solution: 3.452 millimols

Fe_2O_3 in solution: 0.0125 millimols.

Composition of floc: $\text{Fe}_2\text{O}_3(\text{SiO}_2)_{1.39}$.

TABLE 39A

*The AlCl₃ + Na₂SiO₃ + NaOH—System No. 13*A. 5.0 millimols AlCl₃ in 1,000 cc.B. 7.5 millimols Na₂SiO₃ + 15 millimols NaOH in 1,000 cc.

SOLUTION A	SOLUTION B	FLOCCULATION		μ /SEC. 1 VOLT/CM.	pH
		After mixing	Overnight		
cc.	cc.				
20	9.1	Opal	0
20	9.2	Slow	x
20	9.3	Instant	xxxx	+1.38	6.35
20	9.4	Instant	xxxx	+0.87	6.55
20	9.5	Instant	xxxx	— slight	6.7
20	10.0	Opal	0

Isoelectric mixture: $\frac{\text{SiO}_2}{\text{Al}_2\text{O}_3} = 1.43$.

5.0 millimols AlCl₃ in 1,000 cc.3.562 millimols Na₂SiO₃ + 7.124 millimols NaOH in 1,500 cc.

pH 6.6.

SiO₂ in solution: 0.828 millimols.Al₂O₃ in solution: trace.Composition of floc: Al₂O₃(SiO₂)_{1.08}.

TABLE 39B

*The FeCl₃ + Na₂SiO₃ + NaOH—System No. 14*A. 5.0 millimols FeCl₃ in 1,000 cc.B. 7.5 millimols Na₂SiO₃ + 15 millimols NaOH in 1,000 cc.

SOLUTION A	SOLUTION B	FLOCCULATION		μ SEC 1 VOLT, CM.	pH
		After mixing	Overnight		
cc.	cc.				
20	9.3	Opal	0
20	9.4	Instant	xxxx	+1.26	5.7
20	9.5	Instant	xxxx	+0.94	6.0
20	9.6	Instant	xxxx	—0.36	6.45
20	9.8	Instant	xxxx	—1.59	6.65
20	10.0	Opal	0

Isoelectric mixture: $\frac{\text{SiO}_2}{\text{Fe}_2\text{O}_3} = 1.44$.

5.0 millimols FeCl₃ in 1,000 cc.3.588 millimols Na₂SiO₃ + 7.176 millimols NaOH in 1,500 cc.

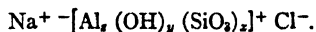
pH 6.35.

SiO₂ in solution: 1.631 millimols.Fe₂O₃ in solution: trace.Composition of floc: Fe₂O₃(SiO₂)_{0.78}.

seen that the position of the two pairs of curves is reversed. The ferric phosphate curve is steeper than that of aluminum and the aluminum silicate curve is steeper than that of the iron. Since $\text{Fe}(\text{OH})_3$ is more insoluble than $\text{Al}(\text{OH})_3$, as shown by the fact that ferric salts are more easily hydrolyzed, it follows from the position of the curves that ferric phosphate is more insoluble than aluminum phosphate. It does not necessarily follow, however, that aluminum silicate is more insoluble than ferric silicate, because the firmer union between Fe and OH would alone result in a lower silicate content. Ferric silicate is therefore more readily hydrolyzed than is aluminum silicate. This is quite evident in the natural soil colloids (16) and explains the formation of ferruginous clays.

As in the case of the "hydroxide" and "phosphate," the "silicate" of iron is isoelectric at a lower pH than the corresponding aluminum compound. In the case of the more highly silicated complexes which are isoelectric at low pH values (between 4.0 and 5.0) this relationship reversed itself, the aluminum complex being isoelectric at the lowest pH.

The preceding isoelectric "silicate" complexes may be represented by the following general formula



This does not mean that the Na ions must equal the Cl ions. It is only the dissociated diffusible cations and anions which must be equal at the isoelectric point. The degree of dissociation may be very different. Also, the preceding formula takes no account of the H ions which are also present in the system and which must, to a large extent, displace the Na ions.

The aforementioned complex may be termed the "chloridated complex" as distinguished from another very illuminating example—the sulfated, silicate complex.

THE SULFATED, SILICATE COMPLEX

In tables 40a and 40b the same concentrations as in tables 38a and 38b were used but instead of the chlorides of iron and aluminum, the sulfates were employed.

A comparison of the composition of the "sulfated silicates" with the corresponding "chloridated silicates" in tables 38a and 38b shows that the sulfated complex is isoelectric with a lower silica content, with a considerably higher proportion of SO_4 than the proportion of Cl in the chloridated complex, and at a lower pH value. This is all in harmony with the preceding observations that the SO_4 ion is more firmly associated in the complex. Since the SO_4 ions are less dissociated than the Cl ion, a greater proportion must be present in order that the anionic dissociation shall balance the cationic dissociation at the isoelectric point. As in the case of the "hydroxide," the isoelectric pH is lower in the sulfated complex.

By displacing a highly dissociating anion like the Cl ion by a less dissociat-

TABLE 40A
The $Al_2(SO_4)_3 + Na_2SiO_3$ —System No. 15
 A. 2.5 millimols $Al_2(SO_4)_3$ in 1,000 cc.
 B. 7.5 millimols Na_2SiO_3 in 1,000 cc.

SOLUTION A	SOLUTION B	FLOCCULATION		μ /SEC. 1 VOLT/CM.	pH
		After mixing	Overnight		
cc.	cc.				
20	8	Slow	xxxx	+1.01	4.1
20	10	Rapid	xxxx	4.2
20	12	Instant	xxxx	+0.68	4.3
20	14	Instant	xxxx	+0.67	4.45
20	16	Instant	xxxx	+0.50	5.1
20	17	Instant	xxxx	+0.42	5.75
20	18	Instant	xxxx	-0.95	6.4
20	19	Opal	x

Isoelectric mixture: $\frac{SiO_2}{Al_2O_3} = 2.60$.

2.5 millimols $Al_2(SO_4)_3$ in 1,000 cc.

6.488 millimols Na_2SiO_3 in 1,500 cc.

pH 6.1.

SiO_2 in solution: 3.866 millimols.

SO_4 in solution: 7.153 millimols.

Al_2O_3 in solution: None.

SO_4 added: 7.500 millimols.

Composition of floc: $Al_2O_3(SiO_2)_{1.06}(SO_3)_{0.159}$ or $Al_2(OH)_2(SiO_3)_{1.06}(SO_4)_{0.159}$.

TABLE 40B
The $Fe_2(SO_4)_3 + Na_2SiO_3$ —System No. 16
 A. 2.562 millimols $Fe_2(SO_4)_3$ in 1,000 cc.
 B. 7.5 millimols Na_2SiO_3 in 1,000 cc.

SOLUTION A	SOLUTION B	FLOCCULATION		μ /SEC 1 VOLT/CM	pH
		After mixing	Overnight		
cc.	cc.				
20	10	Slow	xxx	+0.45
20	12	Rapid	xxxx	+0.36
20	14	Instant	xxxx	+0.28	3.4
20	16	Instant	xxxx	+0.33	4.0
20	17	Instant	xxxx	+0.32	4.8
20	18	Instant	xxxx	-0.44	5.75
20	19	Instant	xxx	-2.20	6.5
20	20	Opal	0

Isoelectric mixture: $\frac{SiO_2}{Fe_2O_3} = 2.54$.

2.562 millimols $Fe_2(SO_4)_3$ in 1,000 cc.

6.525 millimols Na_2SiO_3 in 1,500 cc.

pH 5.4.

SiO_2 in solution: 4.694 millimols.

SO_4 in solution: 7.247 millimols.

Fe_2O_3 in solution: None.

SO_4 added: 7.687 millimols.

Composition of floc: $Fe_2O_3(SiO)_{0.716}(SO_3)_{0.176}$ or $Fe_2(OH)_2(SiO_3)_{0.716}(SO_4)_{0.176}$

ing anion, the isoelectric pH is always displaced in the direction of greater acidity. [Conversely if we displace highly dissociating cations by slightly dissociating cations we will displace the isoelectric pH in the opposite direction. Thus, if we add aluminum or ferric chloride to an isoelectric silicate or phosphate, the system will be electropositive at the same pH, the isoelectric pH being now higher. This fact is of great importance in relation to ion adsorption and exchange. Soil colloids high in aluminum and iron possess a low cation exchange capacity, the Al and Fe not themselves being exchangeable, and these colloids are isoelectric within the usual range of soil reactions (8)].

It is evident that the SiO_3 ion does not displace the more firmly associated SO_4 as readily as it displaces the Cl ion. Although the silicate concentration in the solution at equilibrium is higher and the pH is lower in the sulfate than in the corresponding chloride systems, the number of mols of SiO_2 per mol sesquioxide has decreased from 1.63 in the chloridated to 1.05 in the sulfated aluminum silicate and from 1.29 to 0.715 in the corresponding ferric compounds. But the decrease in the silicate content is only partly accounted for by the SO_4 content. The sulfated silicate complexes are more basic than the corresponding chloridated complexes, indicating a firmer hydroxide union in the former.

At the lower pH values at which the "silicates" are isoelectric, more SO_4 remains in the complex than was found in the isoelectric "hydroxides." This would indicate that the silicate complex contains a higher proportion of dissociating (exchangeable) cations than the hydroxide complex. (This we know to be the case.) A larger proportion of SO_4 ions is therefore necessary in order that the anionic dissociation shall balance the cationic dissociation.

In the absence of silicate, how much SO_4 would be found in the complexes at the same pH values? To answer this question the same number of millimols of $\text{Al}_2(\text{SO}_4)_3$ and $\text{Fe}_2(\text{SO}_4)_3$ were alkalized with NaOH to the same pH values, i.e., 6.1 and 5.4 respectively, and the supernatant liquids analyzed for SO_4 . This procedure is made possible by the fact that in the presence of the divalent SO_4 anion the positive sol is flocculated completely over a wide range as is shown in the tables. The results were as follows:

2.5 millimols $\text{Al}_2(\text{SO}_4)_3$ in 2,500 cc.
 pH 6.1 adjusted with NaOH
 SO_4 in system: 7.500 millimols
 SO_4 in solution: 6.380 millimols
 Composition of floc: $\text{Al}_2\text{O}_3(\text{SO}_3)_{0.448}$ or
 $\text{Al}_2(\text{OH})_{5.104}(\text{SO}_4)_{0.448}$

2.562 millimols $\text{Fe}_2(\text{SO}_4)_3$ in 2,500 cc.
 pH 5.4 adjusted by NaOH
 SO_4 in system: 7.687 millimols
 SO_4 in solution: 6.977 millimols
 Composition of floc: $\text{Fe}_2\text{O}_3(\text{SO}_3)_{0.277}$ or
 $\text{Fe}_2(\text{OH})_{5.446}(\text{SO}_4)_{0.277}$

In the absence of the silicate and at the same pH, the complexes have a higher SO_4 content and are electropositive. In the isoelectric complexes, portions of both of the OH and SO_4 ions have been displaced by the SiO_3 ions.

All the ions in the system mutually displace one another in the colloidal

complex and thus affect the composition and behavior of the complex. The displacing power or association constant of the ions in the systems thus far considered seems to be



But the association constant apparently varies with the composition of the colloidal ion complex, which is quite natural. Since the composition of the complex varies with the nature and concentration of each ion in the solution, no fixed magnitude can be assigned to the displacing power of any ion. This will explain the many discrepancies in the various ion series suggested by different investigators.

The law governing ion exchange may be stated thus: All ions in the solution displace one another in the complex in proportion to the product of their concentration (activity) and the association constant.

EFFECT OF DILUTION ON THE pH AND COMPOSITION OF ISOELECTRIC "SILICATES"

To study the effect of dilution a mixture ratio of $\frac{\text{SiO}_2}{\text{R}_2\text{O}_3} = 10.0$ was maintained in each case. The systems were rendered isoelectric by adjusting the pH by small increments of HCl which was added to the aluminum or ferric chloride before these solutions were mixed with the silicate. Tables 41a and 41b represent the dilute and tables 42a and 42b represent the concentrated systems, the silica/sesquioxide ratio being 10 in each case.

The results are similar to those of the dilution experiments with the phosphate and fit the curves in figure 6 fairly well. The lower the dilution the greater is the number of silicate ions entering into combination with the complex. In the concentrated systems the isoelectric pH is markedly displaced to the more acid side. The highly dissociating Cl ions are here so much more energetically displaced by the higher SiO_3 -ion concentration that a lower pH is necessary to leave a sufficient residue of Cl ion in the complex to balance the cationic dissociation and render the colloid isoelectric. There are, therefore, two factors which, in the more concentrated isoelectric system, lead to a more highly silicated floc. In the first place the silicate-ion concentration is higher, and in the second place the OH-ion concentration is lower; consequently these ions compete less for a place in the complex.

As already has been noted, the isoelectric pH is lower in the aluminum than in the ferric silicate system at low pH values. At higher pH values the order is reversed.

It is significant that even though the equilibrium silicate concentration attains considerable proportions in the concentrated system, the ratio of silica to sesquioxide in the floc approaches, but does not exceed, a value of 3.0, which would be that of the normal silicate. This indicates that the reaction is between single ions, for if it were a combination between colloidal aggregates of sesquioxide and silica as in the mutual flocculation of positive and

TABLE 41A
The dilute $AlCl_3 + Na_2SiO_3$ —System No. 25

A. 2.0 millimols $AlCl_3$ in 1,000 cc.

B. 10.0 millimols Na_2SiO_3 in 1,000 cc.

SOLUTION A 20 cc. + HCl MILLIMOLS	SOLUTION B	FLOCCULATION		μ /SEC. 1 VOLT/CM	pH
		After mixing	Overnight		
	cc.				
0.3000	20	Opal	0
0.3050	20	Instant	xxxx	-1.44	...
0.3075	20	Rapid	xxxx	-0.55	5.6
0.3100	20	Slow	xxxx	+0.61	5.0
0.3200	20	Opal	0

Isoelectric mixture: $\frac{SiO_2}{Al_2O_3} = 10.0$.

2.0 millimols $AlCl_3 + 15.43$ millimols HCl in 1,000 cc.

10.0 millimols Na_2SiO_3 in 1,500 cc.

pH 5.6.

SiO_2 in solution: 7.872 millimols.

Al_2O_3 in solution: 0.016 millimols.

Composition of floc: $Al_2O_3(SiO_2)_{2.16}$.

TABLE 41B
The dilute $FeCl_3 + Na_2SiO_3$ —System No. 26

A. 2.0 millimols $FeCl_3$ in 1,000 cc.

B. 10.0 millimols Na_2SiO_3 in 1,000 cc.

SOLUTION A 20 cc + HCl MILLIMOLS	SOLUTION B	FLOCCULATION		μ /SEC 1 VOLT/CM	pH
		After mixing	Overnight		
	cc.				
0.2950	20	Clear	0
0.3000	20	Rapid	xxx	-1.89	...
0.3050	20	Instant	xxxx	-1.05	...
0.3075	20	Rapid	xxxx	-0.65	5.6
0.3100	20	Opal	xxxx	+0.50	4.4
0.3200	20	Opal	0

Isoelectric mixture: $\frac{SiO_2}{Fe_2O_3} = 10.0$.

2.0 millimols $FeCl_3 + 15.44$ millimols HCl in 1,000 cc.

10.0 millimols Na_2SiO_3 in 1,500 cc.

pH 5.4.

SiO_2 in solution: 8.170 millimols.

Fe_2O_3 in solution: 0.010 millimols.

Composition of floc: $Fe_2O_3(SiO_2)_{1.86}$.

TABLE 42A

*The concentrated $\text{AlCl}_3 + \text{Na}_2\text{SiO}_3$ —System No. 27*A. 7.5 millimols AlCl_3 in 1,000 cc.B. 37.5 millimols Na_2SiO_3 in 1,000 cc.

SOLUTION A 20 cc. + HCl MILLIMOLS	SOLUTION B	FLOCCULATION		μ/SEC 1 VOLT/CM.	pH
		After mixing	Overnight		
	cc.				
1.14	20	Slow	x
1.15	20	Instant	xxx	-1.51	4.6
1.16	20	Instant	xxxx	-1.04
1.17	20	Instant	xxxx	-0.74
1.18	20	Instant	xxxx	-0.25	4.3
1.19	20	Instant	xxxx	± 0.0	4.25
1.20	20	Instant	xxxx	+0.43	4.2
1.22	20	Instant	xxxx	+1.09
1.26	20	Slow	xx	+1.12	4.1

Isoelectric mixture: $\frac{\text{SiO}_2}{\text{Al}_2\text{O}_3} = 10.0$.

7.5 millimols $\text{AlCl}_3 + 59.5$ millimols HCl in 1,000 cc.

37.5 millimols Na_2SiO_3 in 1,500 cc.

pH 4.2.

SiO_2 in solution: 26.840 millimols.

Al_2O_3 in solution: 0.076 millimols.

Composition of floc: $\text{Al}_2\text{O}_3(\text{SiO}_2)_{1.90}$.

TABLE 42B

*The concentrated $\text{FeCl}_3 + \text{Na}_2\text{SiO}_3$ —System No. 28*A. 7.5 millimols FeCl_3 in 1,000 cc.B. 37.5 millimols Na_2SiO_3 in 1,000 cc.

SOLUTION A 20 cc. + HCl MILLIMOLS	SOLUTION B	FLOCCULATION		$\mu/\text{SEC.}$ 1 VOLT/CM.	pH
		After mixing	Overnight		
	cc.				
1.13	20	Opal	0
1.14	20	Instant	xxx	-1.70	...
1.15	20	Instant	xxxx	-1.36	5.0
1.16	20	Instant	xxxx	± 0.0	4.2
1.17	20	Rapid	xx	+0.61	...
1.18	20	Opal	0

Isoelectric mixture: $\frac{\text{SiO}_2}{\text{Fe}_2\text{O}_3} = 10.0$.

7.5 millimols $\text{FeCl}_3 + 58.0$ millimols HCl in 1,000 cc.

37.5 millimols Na_2SiO_3 in 1,500 cc.

pH 4.35.

SiO_2 in solution: 26.593 millimols.

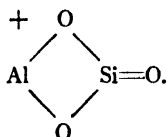
Fe_2O_3 in solution: 0.042 millimols.

Composition of floc: $\text{Fe}_2\text{O}_3(\text{SiO}_2)_{1.94}$.

negative sols, where only the surface layer of molecules are able to react, we could hardly expect such regularity in the composition ratio.

In the higher concentrations the silica shows by itself a tendency to flocculate when sodium silicate is slightly acidified. There is, therefore, the possibility in systems of low dilution of flocculating free silica together with the isoelectric complex. This might have happened in the concentrated ferric system in which the composition ratio of the floc was extraordinarily high. Such complications, which are of course not met with in the phosphate systems, are always present in the "humate" systems as we shall see later, for "humic acid" is still more colloidal than silica.

It will be noted that Fe and, to a higher degree, Al appear in the solution in increasing quantities with a lowering of the pH, but it is doubtful whether they dissociate as free metal cations. The author has established the fact that when orthoclase is electrodialyzed, aluminum appears *together with silica* in the cathode chamber after the pH of the feldspar has been reduced to a certain value by the removal of potassium. It was then suggested that the aluminum and silica exist in acid solution in the form of a complex cation as exemplified by the following formula (9)



The structure of this ion is identical with the structure assumed for the electropositive colloidal silicate complex and is arrived at by the further assumption that the latter is capable of splitting off single such ions or ion aggregates small enough to pass through the membrane. In other words, the positive silicate complex is slightly soluble and the aluminum and iron found in the acid soil solution are not entirely due to a breaking up of the complex. It would be interesting to determine whether the negative complex similarly yields a diffusible aluminum silicate anion when saturated with the strongly dissociating Na ions.

Odén (14) and later Wiegner and Palmann (19) observed silica migrating to the cathode when a soil is electrodialyzed. This fact was originally overlooked by the author but was predicted in connection with the feldspar experiment and has since been verified. That the aforementioned isoelectric precipitates yield a cathodic complex containing silica and sesquioxides will be shown later.

INFLUENCE OF STATE OF DISPERSION OF REACTING MATERIALS ON COMPOSITION OF PRECIPITATES

In the following experiments a mixture ratio of 14 mols silica to 1 mol sesquioxide was maintained throughout. The HCl was added, as before,

TABLE 43A

*The (AlCl₃ + HCl) + Na₂SiO₃—System No. 29*A. 2.5 millimols AlCl₃ in 1,000 cc.B. 17.5 millimols Na₂SiO₃ in 1,000 cc.

SOLUTION A 20 cc. + HCl MILLIMOLS	SOLUTION B	FLOCCULATION		μ /SEC. 1 VOLT/CM.	pH
		After mixing	Overnight		
	cc.				
0.580	20	Clear	0
0.590	20	Opal	0
0.595	20	Rapid	xx	-1.97	5.5
0.600	20	Instant	xxxx	-1.31	5.0
0.605	20	Rapid	xxxx	+0.20	4.6
0.610	20	Slow	xxxx	+0.67	4.4
0.620	20	Opal	xx	+0.82	4.25

Isoelectric mixture: $\frac{\text{SiO}_2}{\text{Al}_2\text{O}_3} = 14.0$.

2.5 millimols AlCl₃ + 30.2 millimols HCl in 1,000 cc.

17.5 millimols Na₂SiO₃ in 1,500 cc.

pH 4.7.

SiO₂ in solution: 14.138 millimols.

Al₂O₃ in solution: 0.052 millimols.

Composition of floc: Al₂O₃(SiO₂)_{2.50}.

TABLE 43B

*The (FeCl₃ + HCl) + Na₂SiO₃—System No. 30*A. 2.5 millimols FeCl₃ in 1,000 cc.B. 17.5 millimols Na₂SiO₃ in 1,000 cc.

SOLUTION A 20 cc. + HCl MILLIMOLS	SOLUTION B	FLOCCULATION		μ SEC. 1 VOLT/CM.	pH
		After mixing	Overnight		
	cc.				
0.590	20	Opal	xx	-1.78	...
0.595	20	Instant	xxx	-1.44	5.8
0.600	20	Opal	xxxx	-0.95	5.0
0.6025	20	Opal	xxxx	+0.28	4.4
0.605	20	Opal	0
0.608	20	Clear	0

Isoelectric mixture: $\frac{\text{SiO}_2}{\text{Fe}_2\text{O}_3} = 14.0$.

2.5 millimols FeCl₃ + 30.09 millimols HCl in 1,000 cc.

17.5 millimols Na₂SiO₃ in 1,500 cc.

pH 4.7.

SiO₂ in solution: 14.619 millimols.

Fe₂O₃ in solution: 0.032 millimols.

Composition of floc: Fe₂O₃(SiO₂)_{3.36}.

TABLE 44A
The $AlCl_3 + (Na_2SiO_3 + HCl)$ —System No. 31

A. 2.5 millimols $AlCl_3$ in 1,000 cc.

B. 17.5 millimols Na_2SiO_3 in 1,000 cc.

SOLUTION A	SOLUTION B 20 cc. + HCl MILLIMOLS	FLOCCULATION		μ /SEC. 1 VOLT/CM.	pH
		After mixing	Overnight		
cc.					
20	0.610	Clear	0	4.3
20	0.615	Opal	xxxx	−1.01	4.25
20	0.620	Slow	xxxx	−0.87	4.15
20	0.630	Opal	xxxx	−0.80	4.10
20	0.640	Clear	xxxx	4.05
20	0.650	Clear	xxxx	−0.76	3.9

Electronegative mixture: $\frac{SiO_2}{Al_2O_3} = 14.0$.

2.5 millimols $AlCl_3$ in 1,000 cc.

17.5 millimols $Na_2SiO_3 + 31.0$ millimols HCl in 1,500 cc.

pH 4.15.

SiO_2 in solution: 5.637 millimols.

Al_2O_3 in solution: 0.105 millimols.

Composition of floc: $Al_2O_3(SiO_2)_{10.85}$.

TABLE 44B
The $FeCl_3 + (Na_2SiO_3 + HCl)$ —System No. 32

A. 2.5 millimols $FeCl_3$ in 1,000 cc.

B. 17.5 millimols Na_2SiO_3 in 1,000 cc.

SOLUTION A	SOLUTION B 20 cc. + HCl MILLIMOLS	FLOCCULATION		μ /SEC 1 VOLT/CM.	pH
		After mixing	Overnight		
cc.					
20	0.595	Clear	0
20	0.5975	Clear	0
20	0.600	Opal	0
20	0.6025	Clear	xxxx	−0.25	4.2
20	0.605	Clear	0
20	0.615	Clear	0

Electronegative mixture: $\frac{SiO_2}{Fe_2O_3} = 14.0$.

2.5 millimols Fe_2Cl_3 in 1,000 cc.

17.5 millimols $Na_2SiO_3 + 30.125$ millimols HCl in 1,500 cc.

pH 4.2.

SiO_2 in solution: 7.235 millimols.

Fe_2O_3 in solution: 0.054 millimols.

Composition of floc: $Fe_2O_3(SiO_2)_{8.89}$.

in small increments, sufficient to cover the whole range of precipitation. The acid was added to the AlCl_3 and FeCl_3 solutions in one set, and to the silicate solution, before mixing, in the second set. This resulted in partly changing the silica to the colloidal condition before it reacted with the Al and Fe. The results are shown in tables 43a and 43b and in 44a and 44b.

In the two experiments where the HCl was added to the chloride solutions before mixing, the composition of the floc is in line with all previous experiments. The points representing the composition ratio and the silicate concentration at equilibrium come in close proximity of the silicate curves in figure 6. It would seem, therefore, that no matter how high the ratio of silica to sesquioxide is in the mixture, an isoelectric floc will form, in which the composition ratio is smaller than 3.0, provided the system is kept sufficiently dilute. In the author's previous experiments (9) the composition ratio already exceeded this value at a mixture ratio of 8.0 but the system, it will be noted, was then more concentrated. There is evidently a critical point at which free silica flocculates together with the complex. The floc has then no isoelectric point but remains negative and in systems of still higher concentration the flocculation becomes incomplete.

The conditions are then similar to those in the last two experiments where the HCl was added to the silicate before this solution was mixed with the chlorides. The acid changes the silica partly to the colloidal condition. Where, under the conditions of true solution, single ions combined, there is now a combination of Al and Fe ions with aggregates of silicate molecules and ions. The ratio of silica to sesquioxides in the resulting complex must therefore be abnormally high. Since the "free" silica in the complex combines with the various diffusible cations in the solution (in this case Na and H) the cationic dissociation will be increased. The complex will therefore be more electronegative and may have no isoelectric point, for although it contains a certain number of diffusible anions (in this case Cl ions) at low pH values, the anionic dissociation may never balance that of the cations.

It will be noted in the tables 44a and 44b that a reduction in the dispersion of the silicate resulted in a floc in which the ratio of silica to sesquioxide is enormously increased and also that the complex remains negative over the entire range of flocculation. The ferric complex shows a very narrow zone of flocculation. This is important, for it shows that silica exerts a protective action on the iron, as definitely shown by the work of Reifenberg (15). In general, ferric "silicates" show a more narrow zone of flocculation than the aluminum "silicates."

INFLUENCE OF DIVALENT CATIONS ON THE ALUMINUM AND FERRIC "SILICATE" SYSTEMS

As long as the monovalent ions, such as the Cl and NO_3 anions and the alkali cations, are the only diffusible ions present in the system, flocculation is confined to a narrow zone on either side of the isoelectric point. We have

already studied the effect of the presence of a divalent anion, i.e., the SO_4 ion. The effect was twofold: The flocculating zone was greatly extended on the positive side of the isoelectric point; and the composition ratio of silica to sesquioxides in the isoelectric complex was markedly reduced (compare tables 40a and 40b).

The divalent cations have already been shown (9) to have the opposite effect to that of the SO_4 ion. In the presence of divalent cations the composition ratio of the isoelectric complex may exceed the ratio of 3. This does not mean that more than 3 mols of silica are in actual combination with one mol of sesquioxide. It is evident that some of the silicate valences are linked with the divalent cations, which accounts for the high ratio. There is evidently also no limit to the amount of excess silica which may thus be fixed in the complex, but once the silica content exceeds a certain proportion, the complex ceases to be amphoteric; it remains negative at all pH values.

The presence of divalent cations also extends the zone of flocculation on the negative side of the isoelectric point. No matter how strongly negative the sol may be it will flocculate at once if sufficient $\text{Ca}(\text{OH})_2$ is added. The silica will then all be carried down in the floc and if the ratio of silica to sesquioxide in the mixture is high the composition ratio in the floc will be equally high and the complex will be strictly electronegative.

If a high proportion of silica be isoelectrically precipitated as in the concentrated systems in tables 42a and 42b, and if, after mixing, the system be diluted and the pH be adjusted to that of the isoelectric value of the dilute systems in tables 41a and 41b, it is found that the composition ratio is intermediate between that of the concentrated and the dilute systems. In other words, the silica, being of a colloidal nature, is not completely reversible. If any isoelectric silicate gel is dried the reversibility is greatly reduced. This will explain the great stability of the natural soil colloids which may have been subjected to alternate drying and wetting and, more important, to the process of aging which leads to a coarser structure and evidently also to a certain degree of crystallization, as shown by X-ray photographs (3).

INFLUENCE OF THE $\frac{\text{SiO}_2}{\text{R}_2\text{O}_3}$ RATIO ON THE ADSORPTIVE AND ELECTROKINETIC
BEHAVIOR OF THE NATURAL SOIL COLLOIDS

Two tables which have appeared in a previous publication (8) are given here in a condensed form. Table 45 shows the adsorption of the PO_4 , SO_4 , and Cl ions from solutions of the respective acids by a series of soil colloids of varying silica/sesquioxide ratio. The charge and speed of electrical migration are also shown. Table 46 shows the adsorption of the same ions from a mixture of the acid with increasing proportions of ammonia, by the Norfolk soil colloid.

For a detailed discussion of these experiments the reader is referred to the

original paper. For our present subject the following points of comparison are of interest:

The anion adsorption increases with a decrease in the silica/sesquioxide ratio. The increase in adsorption is greater than the absolute increase in the sesquioxides per gram colloid. This indicates that the silica and sesquioxides exist in a more or less stable combination. Colloids very high in silica, like the Bentonite and Fallon, do not adsorb any

TABLE 45

Adsorption of the PO₄, SO₄, and Cl ions from solutions of the respective acids by different soil colloids

Initial concentrations: H₃PO₄, 1.768; H₂SO₄, 1.624; HCl, 1.640; milliequivalent in 100 cc.

COLLOID 1 GM.	SiO ₂ R ₂ O ₃	PO ₄		SO ₄		Cl	
		Ad- sorbed milli- equiv- alent per gram	μ/sec 1 volt/ cm.	Adsorbed milli- equiv- alent per gram	μ/sec 1 volt/ cm.	Adsorbed milli- equiv- alent per gram	μ/sec. 1 volt/ cm.
Bentonite.....	3.81	0.214	-2.3	-0.005	N.D.	N.D.	N.D.
Fallon.....	3.82	0.518	-1.0	-0.003	-1.1	-0.004	-0.6
Sharkey.....	3.18	0.700	-1.3	0.036	-0.8	0.004	-0.4
Marshall.....	2.82	0.932	-0.7	0.041	-0.7	Lost	-0.2
Sassafras.....	1.89	1.152	+0.2	0.151	-0.2	0.034	+1.4
Norfolk.....	1.63	0.904	+0.6	0.129	-0.1	0.026	+1.7
Aragon.....	0.55	1.601	+1.3	0.269	+0.6	0.044	+2.8

TABLE 46

Adsorption of the PO₄, SO₄, and Cl ions from solutions of the respective acids, with various additions of ammonia, by the Norfolk soil colloid

Initial concentrations: H₃PO₄, 6.14; H₂SO₄, 5.41; HCl, 5.05; milliequivalent in 50 cc.

NH ₃ ADDED MILLIEQUIV- VALENT IN 50 cc	PO ₄			SO ₄			Cl		
	Adsorbed milliequiv- alent per gram	pH	μ sec 1 volt cm	Adsorbed milliequiv- alent per gram	pH	μ/sec. 1 volt/ cm.	Adsorbed milliequiv- alent per gram	pH	μ sec. 1 volt, cm
0.0	0.84		+1.1	0.164		-0.5	0.079		N.D.
1.287	0.88	<4.4	-0.9	0.163		-0.9	0.075		+1.7
2.574	0.56	6.2	-3.5	0.121		-1.2	0.060		+1.4
3.861	0.42	6.8	-3.6	0.096		-1.4	0.037		+1.1
5.148	Lost	>8.0	-3.8	0.071	<4.4	-1.5	0.017	<4.4	+0.1
7.722	0.27		-4.1	-0.031	>8.0	-3.6	-0.019	>8.0	-3.3

sulfuric or hydrochloric acid. (Where there is no positive adsorption a negative adsorption due to the Donnan distribution is observed.) All of the sesquioxides are apparently here in combination with the silica.

The PO₄ is much more strongly adsorbed than the SO₄ ion and the SO₄ more strongly than the Cl ion. The relative order of magnitudes is not very different from that observed in the isoelectric precipitates. The Bentonite and the Fallon colloids, which adsorb neither SO₄ nor Cl ions, adsorb considerable quantities of the PO₄ ion. Whether this is due to a

displacement of silica or to the formation of an addition compound, or both, will be discussed in a later section.

The quantities of the various ions adsorbed decrease with an increase in pH, the PO_4 ion being the only ion which is adsorbed from an alkaline solution.

Soil colloids having a low silica/sesquioxide ratio and adsorbing the greatest quantities of anions become electropositive in acid solutions. The isoelectric point lies at a higher pH the higher the sesquioxide content. The relationship is therefore the same as that found to govern the isoelectric precipitates. The divalent SO_4 ion is not as highly dissociated by the complex as the monovalent Cl and H_2PO_4 ions. (At low pH values the phosphate ion is chiefly monovalent). The positive charge therefore attains higher values in the H_3PO_4 and HCl solutions than in that of the H_2SO_4 . The same phenomenon was met with in the foregoing synthetic sols.

FACTORS GOVERNING THE COMPOSITION OF THE INORGANIC SOIL COLLOIDS

The lithosphere has, according to Clarke (1), the following average composition: SiO_2 , 59.85; $\text{Al}_2\text{O}_3 + \text{Fe}_2\text{O}_3$, 21.35; CaO , 4.81; MgO , 3.77; K_2O , 3.02; and Na_2O , 3.29 per cent. If the Fe_2O_3 is assumed to constitute one-fourth of the total sesquioxides, a silica/sesquioxide ratio equal to 5.2 is reached. This is higher even than that of the highest soil colloid ratios but since some of the silica (about 12 per cent) exists in the form of highly insoluble quartz we cannot expect such a high ratio in the colloids.

When rocks weather under arid conditions, there is little or no loss of material. Most of the products of hydrolysis remain in the soil and react with one another to form a colloidal complex, doubtless in accordance with the same laws we have found to govern the formation of the complexes described in the foregoing chapters. The sesquioxides will combine with a certain proportion of the silica and, in the presence of an abundance of divalent bases, the remainder of the silica will be precipitated by them. The complex will therefore have a high silica/sesquioxide ratio and be a strictly electronegative complex. It will owe its stability to the presence of the divalent cations. Soil colloids high in silica and bases occur chiefly in arid regions, as shown by the very valuable work of Robinson and Holmes (16).

Suppose, for the sake of continuity in our line of reasoning, that the aridity is changed into great humidity. Under such conditions, what would happen to the colloidal soil complex would indirectly depend upon another dominating factor, namely, the temperature. Whether in the tropics or in the cold regions, the soil would be subjected to great leaching, and the bases, and with them the protected silica, would be progressively washed out. In the colder regions there would be an accumulation of organic matter and the consequent development of a high soil acidity, but this would not happen in tropical regions. In the tropics there is no accumulation of humus. This, together with more intense weathering, keeps up a small but constant supply of bases, and maintains, in spite of the leaching, a more nearly neutral reaction.

The acid reaction on the one side and the more nearly neutral reaction on the other would have a profound influence upon the composition of the colloidal complex, if we can draw any inference from the foregoing study.

It has been shown that in the absence of divalent bases the sesquioxides precipitate with silica a complex which is richer in sesquioxides and poorer in silica, the higher the pH. We should evidently, therefore, expect to find the soil colloids in the humid, tropical regions possessing a low silica/sesquioxide ratio whereas the soil colloids in the temperate and cold, humid regions, where the soil reaction is more acid (evidently in the absence of carbonates), would be expected to possess a higher silica/sesquioxide ratio. Thus we know that the laterites which contain very little silica are the result of tropical, humid weathering. The work of Robinson and Holmes (16) seems to bear out the second point.

The silica/sesquioxide complex is both chemically and electrokinetically an amphoteric complex. It combines with acids and becomes thereby electro-positive because in this condition it dissociates diffusible anions. It combines with bases and becomes thereby electronegative because in this condition it dissociates, diffusible cations. It follows that, if by leaching, dialysis, or otherwise, the diffusible ions be removed, the complex will approach or attain the isoelectric condition. This condition represents, therefore, the ultimate and the most stable condition. It has been pointed out that colloids are most easily "purified" at the isoelectric point. Conversely if an amphoteric colloid be "purified" it will be brought nearer its isoelectric point. Since the silica/sesquioxide ratio in the isoelectric aluminum and ferric "silicates" is higher the lower the pH, and vice versa, we arrive at the very important deduction that the most stable, and therefore the ultimate, composition of the soil complex will depend upon the pH of the leaching soil solution. The more acid the soil solution the greater will be the proportion of silica remaining precipitated with the sesquioxides after the bases and a portion of the silica have been removed by leaching.

It has been noted by Zakharov (21) that desilication is more rapid and extensive in the humid, tropical regions than in the humid, temperate regions, resulting in the former in the formation of laterites and in the latter in the formation of clays. The presence of the earth carbonates will, under all conditions, delay the desilication because these substances will maintain a saturated and stable condition of the negative complex.

An attempt was made in the foregoing section (11) to represent the size of the colloidal particle as an equilibrium condition between two sets of opposing forces. The higher the exchange capacity and the higher, therefore, the micellar ion density, the greater, it was concluded, must be the degree of dispersion. Since the exchange capacity decreases with the silica/sesquioxide ratio we should expect a lower dispersion, i.e., a larger particle size, and a less plastic and an, all around, less colloidal condition of the complex, the greater the degree of desilication. This is also the case, for the laterites are known to be non-plastic and very difficult to disperse. They also have a tendency to form non-dispersible concretions. This is not surprising when it is considered that their silica content is often so low that their isoelectric point lies

well within the prevailing range of pH values. At the isoelectric point the particles have no ion atmosphere and carry therefore no charge and no water of osmotic hydration. Stripped of the forces by virtue of which the particles can exist as independent micellae, there is nothing to prevent them from cohering into larger and larger units.

Whereas the formation of laterite, and also bauxite, must be the result of the removal of bases and silica from the soil complex under approximately neutral conditions, the formation of kaolinite must result from the same process under acid conditions. For one mol of the Al_2O_3 to retain two mols of SiO_2 after the removal of all cations would require a fairly high acidity. At the high degree of dilution of the soil solution the pH would doubtless have to be below 5. Kaolinite is itself isoelectric between the pH values of 4 and 5 (9). Kaolinite, which is coarsely crystalline as compared to soil colloids and which contains practically no bases, may be assumed to be formed when a colloidal complex of the composition $\text{Al}_2\text{O}_3 \cdot 2 \text{SiO}_2$ is subjected for a great length of time to a hydrogen-ion concentration at or near that of the isoelectric pH of the complex. That kaolinite formation is connected with an acid medium is brought out by the work of Wüst (20), who ascribes it to the action of humus, and by Rösler (19), who assumes it to be a result of pneumatolytic action, i.e., acid gases.

Hydrous aluminum silicates containing a higher proportion of silica, such as beidellite and scolecite with a silica/sesquioxide ratio *about* 3 and pyrophyllite and Montmorillonite with a ratio from *about* 4 to 5 (18), contain always a certain proportion of bases which usually increases with the proportion of silica. These materials have not been desilicated, that is, they have not been degraded by leaching to any great extent, as is indicated by the presence of readily exchangeable cations. It is evidently because of the presence of bases, especially of the divalent cations, that a complex with such high proportions of silica could precipitate out in the first place. Left more or less unaltered for a long time, crystallization has progressed more or less extensively, giving rise to compounds of definite composition and to a greater stability. Some of the bases are locked up within the crystals and become non-exchangeable. (Only about one-third of the bases present in the natural materials are readily exchangeable.)

THE LAW OF DEFINITE PROPORTIONS

The definiteness in composition reported for these hydrous silicates, even when "purified," should not be uncritically accepted. Their composition is as indefinite as their nomenclature. Within the crystal lattice the law of definite proportions doubtless holds. On the surface (and the surface is great in highly dispersed materials) there is no such definiteness. Every ion in the dispersion medium enters into a very complicated exchange equilibrium. The hydroxyl, the phosphate, the "humate," the sulfate, and a number of other anions may displace the silicate ion. Iron displaces aluminum and

vice versa. Every cation in the solution displaces any cation in the complex according to the activity of each. The more highly dispersed the crystalline material is, the more indefinite its composition. Then, crystallization is often very incomplete. Highly insoluble materials, like most colloids, are slow to crystallize and can do so only to the extent that ions which will fit into the crystal lattice are available. It is obvious that a highly heterogenous complex cannot crystallize. When crystallization does take place there must be a change in the composition. The proper ions are selected and the ions which do not fit are rejected. Some of the "adsorbed" ions pass into solution. This explains the reversion of adsorption as a result of aging as first observed by Freundlich and Hase (2).

CHARGE AND CRYSTALLIZATION

The highly silicated, electronegative soil colloids which contain a high proportion of displaceable cations are very fine-grained. Yet it has been shown that they are at least partly crystalline. Since the solubility decreases with an increase in crystal size by virtue of a decreased curvature we might wonder why it is that the crystalline material in soil colloids attains only submicroscopic dimensions. This may be due to the heterogenous makeup of the adsorption layer. It has been shown by Marc (7) that adsorbed ions of a different kind retard crystallization, but it seems probable that the charge resulting from dissociation limits the dimensions of the crystals just as it places a limit on aggregation; crystallization being in effect nothing but an orderly aggregation of ions. Crystallization might also be looked upon as an adsorption of ions which together fit into the symmetrical arrangement characteristic of the crystal lattice. If crystallization is to proceed, the rate of adsorption of the two ions must be equal. If one ion is very large, and, because of a low self-potential, is incapable of an independent existence, while the other ion has a great solution stability and remains dissociated, a noncrystalline, highly charged micelle (as in a soap solution) will result. If the two ions enter the lattice with about equal energy, the charge will be small and the crystallization can proceed unimpeded. If the two ions enter with different energy, one of the ions remaining dissociated in considerable excess, the charge will be high and the most adsorbable ion will be entering the lattice against a steep potential gradient. Why a crystal may form under such conditions and yet remain very small in size as in the case of soil colloids is brought out by the following.

Assuming a potential difference $\zeta = 70$ millivolts, a thickness of the double layer $\delta = 5\mu\mu$ and a dielectric constant $D = 81$, von Hevesy (4) calculates the charge e for particles of different radii from the formula:

$$e = \frac{\zeta D r (r + \delta)}{\delta}$$

and obtains the figures shown in the first and second columns in table 47. By dividing the number of electronic charges on each particle by the square of the radius we get the values in the third column, which represent the relative number of charges per unit surface, or the ionic density on the different size particles.

If crystallization cannot proceed against a potential gradient above a certain maximum we might easily explain why a crystal will form and grow to a certain small size and then no more. On very small particles the ionic density required for the usual maximum potential of 70 millivolts is very great, greater probably than the dissociation. The smallest crystal rudiments would therefore, like very large ions, have a low potential. The crystal would grow until the potential attained the critical value. Needle-shaped and micaceous crystals having points of high curvature might, however, grow indefinitely. Concave areas could not grow, because here the potential would reach a maximum at very slight dissociation. As the dissociation is probably different on different crystal faces the growth would vary accordingly. In concen-

TABLE 47

Number of ions and ion density necessary to charge particles of different size to the same potential

r $\mu\mu$	CHARGE OF PARTICLE IN NUMBER OF ELECTRONS	CHARGES PER UNIT SURFACE OR RELATIVE ION DENSITY
1	6	6.00
2	14	3.50
10	120	1.20
24	550	0.95
100	8,550	0.85
240	47,000	0.82

trated solutions crystals may grow to large dimensions because here the potential is annihilated by the high ion concentration. Very soluble salts accordingly form large crystals.

The quantitative relationship in table 47 may be qualitatively demonstrated, as in figure 7. It is obvious that to produce the same density in the ion atmosphere a greater number of ions must dissociate per unit surface (a) the greater the curvature, i.e., the smaller the particle, since the dissociated ions must then diffuse into a conical expansion of space which becomes greater as the radius of the particles becomes smaller. It should be pointed out that if a greater average thickness δ of the double layer is assumed, which would be justified on the basis of the r_1/r ratios in table 12 (part I), the difference in ion density necessary to give the same potential for large and for very small particles would become very much greater than the difference shown in table 47.

If the degree of dissociation is limited, then the ion density over a very curved surface may never attain the value it will over a less curved surface, i.e., around a larger particle. Assuming this to be the case it can be shown

that the dissolved (free) ions in the solution will be in higher concentration within the less dense micellar ion atmosphere over a highly convex surface than in the more dense ion atmosphere over a less convex surface. This follows from the Donnan equilibrium equation (see part I) which, for a uni-univalent compound, is

$$x_2 = y(y + z)$$

Where the value of z (i.e., the micellar ion concentration) is small, y (the free ion concentration within the atmosphere) will obviously not be so much smaller

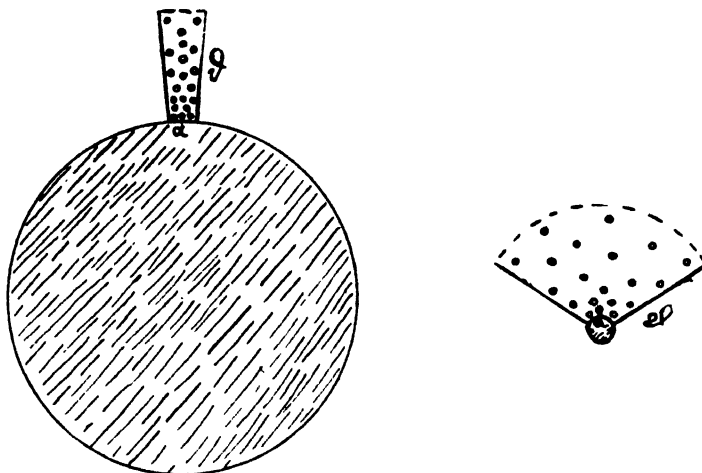


FIG. 7. THE RELATION BETWEEN PARTICLE SIZE OR CURVATURE AND THE ION DENSITY IN THE MICELLAR ATMOSPHERE AT THE SAME DEGREE OF DISSOCIATION PER UNIT SURFACE

than x (the outside ion concentration) as where the value of z is larger. This means further that the Donnan potential

$$\text{P.D.} = \frac{58}{2} \log \left(1 + \frac{z}{y} \right)$$

will be lower between the less dense ion atmosphere and the outside solution than between this solution and the more dense ion atmosphere over a less convex or plane surface. Both of these factors would favor a more rapid deposition of the ions in the solution on surfaces of very great convexity. The higher the valence of the ion with the same sign of charge as that of the interface the greater would be this effect.

If the degree of dissociation is limited then very small particles should show a slower cataphoresis than the larger. This has never been proved and probably never will even if the assumption is correct, because such particles can only have a transient existence, for being unstable they must grow to larger dimensions. To what extent the electrokinetic potential influences the growth of

crystals must be left undecided, the author has merely suggested the possibility of such an influence.

SUMMARY

Isoelectric precipitates of aluminum and ferric "hydroxides" were prepared from the respective chlorides and sulfates. The sulfated hydroxides are isoelectric at a lower pH than the chloridated complex. The SO_4 content of the former is higher than the Cl content in the latter. The ferric complex is isoelectric at a lower pH than the aluminum complex.

The entrance of the phosphate and silicate ions into the sesquioxide complex lowers the isoelectric pH because these ions displace the diffusible acid anions as well as the hydroxyl ions. There are many isoelectric "phosphates" and "silicates." The more highly phosphated or silicated the complex the lower is the isoelectric pH. The lower the phosphate or silicate concentrations in the solution the greater is the relative proportion entering into combination. In high concentrations of the phosphate and silicate ions the isoelectric complex approaches but never attains full saturation, i.e., $\text{P}_2\text{O}_5/\text{Al}_2\text{O}_3 = 1$ and $\text{SiO}_2/\text{Al}_2\text{O}_3 = 3$, provided that other cations which form insoluble phosphates and silicates are absent, and that colloidal silica is not precipitated with the complex.

All the ions in the system mutually displace one another according to the energy of each. At the isoelectric point there is always a certain small number of diffusible anions in combination. At that point the anionic and cationic dissociation must be equal. There is a balance between cations and anions in the complex, but a stoichiometrical relationship between any two ions must be accidental and should not be looked for even when the colloid is "purified." This applies to the more or less crystalline natural colloids as well, for although the law of definite proportions must hold as far as the interior of the crystal is concerned, there is no such definiteness on the surface, which is very great in the colloids. The adsorption layer is very complex in its make-up and changes constantly with the conditions, such as the nature and concentrations of the ions in the solution, the pH, and the temperature.

The work herein described will serve to explain the occurrence of the highly silicated and base-saturated soil colloids in the arid regions, the moderately silicated and more or less base-unsaturated soil colloids in the temperate and colder humid regions, and the almost completely desilicated and base-unsaturated soil colloids in the humid tropical regions.

Another paper (part IV) dealing with the isoelectric aluminum and ferric "humates" is now being prepared.

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